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IODINE METABOLISM IN HYDATIDIFORM MOLE AND CHORIOCARCINOMA*†

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ABSTRACT

Thyroid function and the binding of thyroxine in serum were assessed in 2 women with choriocarcinoma and 3 with hydatidiform mole. Women with choriocarcinoma who were excreting chorionic gonadotropin did not exhibit estrogenic effects or altered binding of thyroxine by the thyroxine-binding globulin. Chorionic gonadotropin *per se* thus appears to play no part in the increase in serum thyroxine-binding characteristic of normal pregnancy. In women with hydatidiform moles, the production of thyroid hormone, as estimated by the thyroidal accumulation of I^{127} and concentrations of serum protein-bound I^{131}, protein-bound I^{127} and butanol-extractable I^{131}, reached values found in thyrotoxicosis. These parameters returned to normal in each case after removal of the pathologic conceptus.

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HYPERPLASIA of the thyroid gland, increased thyroidal accumulation of radioactive iodide, and an increase in the concentration of circulating thyroid hormone develop early in human pregnancy and persist briefly into the puerperium (1-4). The genesis and physiologic significance of these changes remain obscure, and their clarification has been hampered in part by reluctance to administer radioactive isotopes to normally pregnant women. However, unique opportunities for study of the effects of gestation on the metabolism of iodine are provided by the pathologic pregnancies, hydatidiform mole and choriocarcinoma. The present work is concerned with an exploration of the effects of these disorders upon thyroidal economy.

Clinical urgency for treatment of the abnormal pregnancy precluded any studies of iodine metabolism requiring more than a few days of observation. However, within that brief time interval, it was hoped to learn if there was evidence of 1) increased thyroidal accumulation of iodide, 2) increased production and release of hormone from the gland, and 3) alterations in the manner in which thyroxine is bound within the circulation.

MATERIALS AND METHODS

Daily thyroidal accumulation of I^{127} was estimated in 4 of the 5 patients by taking advantage of the assumption that, after mixing, the distribution of I^{131} between thyroid and urine at any time under steady-state conditions is proportional to the distribution of I^{127} between these compartments. Discussions of the validity of the method, description of the techniques employed and assessment of the relationship between thyroidal accumulation of I^{127} and production of thyroid hormone are reported in detail elsewhere (5–7). Briefly, the following method was employed. After an oral dose of inorganic I^{131} (90–100 μc.), the accumulations of thyroidal and urinary I^{131} were assessed on two successive days and expressed as percentages of the administered dose. The thyroidal increment in the accumulation of I^{131} during the second day was calculated by subtracting the 24-hour percentage uptake from the 48-hour value. Stable iodine analyses were performed on aliquots of each daily urine sample and total daily urinary excretion of I^{127} was calculated. Thyroidal accumulation of I^{127} was calculated as follows:

$$\text{Daily thyroidal accumulation of } I^{127}\ (\mu g.I) = \frac{\text{Daily thyroidal increment in } I^{131}\ (\% \text{ administered dose})}{\text{Urinary increment in } I^{131}\ (\% \text{ administered dose}/24\text{-hr. vol.})} \times \text{Daily urinary } I^{127}\ (\mu g.I).$$

Concentrations of serum protein-bound I^{131} (PBI^{131}) and butanol-extractable I^{131} (BEI^{131}) were measured at twenty-four and forty-eight hours following administration of the tracer dose by methods previously described (8, 9). Briefly, concentrations of SPI^{131} were measured by precipitation of the proteins from 3.0-ml. aliquots of serum to which carrier stable thyroxine was added with an equal volume of 10 per cent trichloracetic acid (TCA) followed by multiple washings of the precipitate with 6.0 ml. of 5 per cent TCA. The washed precipitate was dissolved in 3.0 ml. of 10 per cent sodium hydroxide and counted in a well-type scintillation counter.

Concentrations of BEI^{131} were measured by repeated extraction of acidified 3.0-ml. aliquots of serum to which carrier stable thyroxine was added with water-saturated butanol followed by multiple washings with alkaline sodium carbonate (Blau's solution). The pooled extracts were dried *in vacuo*, taken up in 3.0 ml. of butanol and counted in the scintillation counter. The radioactivity of samples prepared for PBI^{131} and BEI^{131} analyses were compared with that of a suitably diluted aliquot of the administered dose.

Aliquots of serum obtained during each period of study were stored in the frozen state for subsequent measurement of the concentration of PBI^{127}, and for assay of thyroxine-binding.

Thyroxine-binding in sera from Patients A.B. and M.W. was studied prior to development of the method currently in use in this laboratory. The earlier method (10) allowed analysis of the binding activity of the thyroxine-binding globulin (TBG) but the absolute binding capacity could not be determined, nor could binding by the newly described thyroxine-binding pre-albumin (TBPA) be assessed (11). The current method was employed in study of Patients D.P., D.E. and P.G. This consisted of zonal electrophoresis of sera in trishydroxymethylaminomethane-maleate buffer (TRIS-maleate), pH 8.6, ionic strength = 0.1 Sera were first enriched with constant quantities of radiothyroxine and increasing quantities of stable thyroxine. After electrophoresis, strips were scanned and peaks of radioactivity integrated by means of an electronic analog integrator (12). Saturation of TBG and TBPA was assumed when, with increasing concentrations of added thyroxine, no further increase occurred in the absolute quantity of thyroxine bound to the binding protein.

Excretion of estrogens was not measured in the patients. Neither mammary engorgement and tenderness nor areolar pigmentary changes were observed, however, in the women with choriocarcinoma.

CASE MATERIAL

Two women with choriocarcinoma and 3 with hydatidiform mole were studied. Abstracts of their case histories were as follows:

Case 1

D.E., a 27-year-old white female, was referred to the Boston City Hospital for treatment of a metastatic choriocarcinoma which followed an entirely normal first pregnancy. Three months after panhysterectomy, chorionic gonadotropin was detected in the urine, and a small dense area in the posterior portion of the left lower lung field was seen on x-ray examination.

At the time of admission to the hospital, the findings on physical examination were not contributory. The thyroid was not enlarged. Using the male *Rana pipiens* test for chorionic gonadotropin, the result was positive with a urine dilution[1] of 1:2,500. Following studies of the patient's thyroid function, she was given 25 mg. of methotrexate (4-amino-N^{10}-methyl pterolyglutamic acid) intravenously, and 20 mg. of the drug daily thereafter for five days. Four weeks later serum was obtained for measurement of the

[1] Highest dilution tested in the assay. Because of the wide seasonal variation in the responsiveness of the male *Rana pipiens* and the lack of comparisons with standard preparations of chorionic gonadotropin, these values are not convertible to international units, and therefore do not have great quantitative significance. However, they equalled values found during normal pregnancy of similar duration, in tests run at the same time of year.

concentration of PBI and for assay of thyroxine-binding by TBG and TBPA. Other studies of thyroid function could not be repeated.

Case 2

D.P., a 25-year-old white female with a history of one previous normal pregnancy, was admitted to the Boston City Hospital for panhysterectomy. Eighteen months prior to admission, she had undergone hysterotomy for a hydatidiform mole. Pathologic examination revealed slight anaplasia of the trophoblast. Following surgery, the patient had normal menses until six months before admission, when amenorrhea developed. A diagnosis of intrauterine pregnancy was made. However, two months later, because of vaginal bleeding and failure of the uterus to increase in size, dilation and curettage were performed. The histologic diagnosis was proliferative endometrium. Subsequently a history of hemoptysis was obtained and chest roentgenograms revealed bilateral "cotton-wool" lesions. The thyroid was slightly and symmetrically enlarged. The uterus was not enlarged. On admission, chorionic gonadotropin was present in the urine in a dilution of 1:1,500. After panhysterectomy and removal of choriocarcinomatous tissue, results of the A-Z test became negative. The patient was given a course of treatment with methotrexate. At the time of readmission six months later the A-Z test yielded negative results, and the pulmonary lesions were no longer visible on x-ray examination. The earlier studies of thyroid function were repeated.

Case 3

A.B., a 30-year-old white female, was admitted to the Boston Lying-In Hospital during her second pregnancy. The patient had appeared to be normally pregnant until the sixteenth week, when vaginal bleeding developed. The size of the uterus was consistent with her dates. Because of persistent bleeding she was admitted during the eighteenth week of pregnancy.

The findings on physical examination were unremarkable, except that no fetal parts could be identified. There was no thyromegaly. Results of the *Rana pipiens* test for chorionic gonadotropin were positive in a 1:2,000 dilution of urine. An abdominal roentgenogram did not show any fetal skeleton.

Studies of the patient's iodine metabolism were performed. After hysterotomy, histologic examination of the mole revealed degenerating trophoblast with small areas of proliferation. On the eighth postoperative day, radioiodine studies were repeated.

Case 4

P.G., an 18-year-old white woman, was referred to the Boston Lying-In Hospital because of a suspected hydatidiform mole. By the sixth week of pregnancy, edema of the ankles and mild hypertension had been noted. Because of rapid increase in uterine size, chronic vaginal bleeding and persisting hypertension the patient was referred for admission during the sixteenth week of pregnancy. There was no past history of renal disease or hypertension, and although 2+ proteinuria was noted, the serum concentration of nonprotein nitrogen, and the excretion of phenolsulfonphthalein were normal.

On admission the patient was a pale, anxious-appearing and hyperkinetic young woman with a pulse of 140 and blood pressure of 150/90 mm. Hg. There was peripheral and facial edema. The heart was not enlarged. The thyroid was palpable, although of normal size; no bruit was heard. The skin was warm and sweaty. No digital tremor was noted. Abdominal examination revealed a uterus consistent in size with pregnancy of twenty-five weeks' duration. There were no fetal heart sounds, and no fetal parts could

be detected. Laboratory data were not remarkable, except for a hematocrit of 27 per cent and 2+ proteinuria. In an abdominal roentgenogram, no fetal skeleton could be discerned. Chorionic gonadotropin was detected in a 1:2,500 dilution of urine.

Studies of thyroid function were completed, and on the sixth hospital day a large mole was removed by hysterotomy. Histologically, areas of myometrial invasion by trophoblastic elements of the mole were noted.

Six weeks later, the Aschheim-Zondek test yielded negative results, there was no radiologic evidence of metastases, and uterine involution was satisfactory. Studies of iodine metabolism were repeated.

Case 5

M.W., a 23-year-old Negress[2] was admitted in the fourteenth week of her third pregnancy with a four-week history of nausea, vomiting, vaginal bleeding and loss of weight. Five years earlier her first pregnancy terminated during the fifth month in the spontaneous delivery of a transitional mole. Her second pregnancy, eighteen months later, terminated at fifteen weeks in a hysterotomy for a second hydatidiform mole. During the latter pregnancy, signs of pre-eclampsia developed and the basal metabolic rate was found to be +41 per cent.

Examination at the time of admission revealed an irritable, nervous, emaciated woman with a blood pressure of 140/70 mm. Hg and a pulse rate ranging from 100 to 140. The uterus was at the level of the umbilicus and appeared consistent in size with pregnancy of twenty to twenty-two weeks' duration. The thyroid gland was palpable but not enlarged. A bruit over the gland disappeared after transfusion of 5 units of blood. No fetal heart sounds were heard, and an abdominal x-ray film failed to demonstrate a fetal skeleton. The BMR was +28 per cent. The concentration of serum PBI was 20.5 μg. per 100 ml. prior to transfusion. Concentrations of serum electrolytes and nonprotein nitrogen were normal. The hematocrit on admission was 27 per cent.

During interruption of this molar pregnancy, both ovaries were found to be markedly enlarged by lutein cysts and the size of the left adrenal was twice normal. The right adrenal was not visualized. The concentration of serum PBI six days after surgery was 11.9 μg. per 100 ml. Six weeks later, no chorionic gonadotropin was detected in the urine.

RESULTS

The data on each patient are presented in Table 1.

Choriocarcinoma

Neither of the patients with the diagnosis of choriocarcinoma exhibited stigmata of pre-eclampsia, or peripheral estrogenic effects. In both, chorionic gonadotropin activity was demonstrable in the urine immediately prior to treatment, and was not detected during the follow-up studies.

It was not possible to repeat the radioiodine studies in Patient D.E., but the initial data revealed increases to the upper limit of the normal range for the 24-hour thyroidal uptake of I^{131} (47.6 per cent of the administered dose), and increases beyond the normal range for the concentration of

[2] It is of interest that this woman had 3 consecutive hydatidiform molar pregnancies, the last with a different husband.

TABLE 1. SEVERAL PARAMETERS OF IODINE METABOLISM IN PATIENTS WITH CHORIOCARCINOMA OR HYDATIDIFORM MOLE

Diagnosis	Patient	Result of A–Z test	Interval after treatment (wks.)	Thyroid			Serum					Serum thyroxine-binding capacity		Thyroxine-binding (earlier method)		
				24-hr. I^{131} uptake (% dose)	Calculated I^{127} incorporation (μg. I/day)		PBI (μg./100 ml.)	PBI^{131} (% dose/L.)		BEI^{131} (% dose/L.)				conc. of added thyroxine per 100 ml.		
					Day 1	Day 2		24 hrs.	72 hrs.	24 hrs.	72 hrs.	TBG* (μg. T_4/100 ml.)	TBPA* (μg. T_4/100 ml.)	10.0 μg.	47.5 μg.	85.0 μg.
Choriocarcinoma			(after methotrexate)													
	D.E.	pos.	—	47.6	230	167	8.9	0.07	0.08	0.04	0.06	24	115			
		neg.	4	—	—	—	7.0	—	—	—	—	—	—			
	D.P.	pos.	—	36.0	110	132	5.3	0.04	0.07	0.03	0.05	21	50			
		neg.	6	39.0	44	54	5.6	0.03	0.05	0.03	0.05	24	90			
Hydatidiform mole			(after surgery)													
	P.G.	pos.	—	81.7	2,146	3,417	13.5	0.13	0.11	0.10	0.10	150	22			
		neg.	6	38.8	104	88	6.9	0.04	0.05	0.03	0.05	40	100			
	A.B.	pos.	—	42.9	84	116	9.0	0.09	0.06	0.06	0.06	—	—	88.4†	71.6	54.3
		neg.	1	31.5	47	58	7.2	0.06	0.06	0.04	0.05	—	—	79.7	64.2	47.1
	M.W.	pos.	—	—	—	—	20.5	—	—	—	—	—	—	88.2	84.0	85.5
			1	—	—	—	11.9	—	—	—	—	—	—	—	—	—
		neg.	7	32.0	—	—	5.0	—	—	—	—	—	—	80.1	56.6	49.8
Normal pregnancy (mean and standard deviation or range)							7.9±1.4					74–100	90–120	90.4 —	76.2 —	57.1¶ —
Normal nonpregnant controls				33.0 ±6.0	70 ±35‡		6.0 ±1.0	0.01–0.03§	0.01–0.06§	0.02 0.01	0.04 0.02	18–30	90–100	76.6 —	51.8 —	50.7¶ —

* TBG = thyroxine-binding globulin; TBPA = thyroxine-binding pre-albumin; T_4 = thyroxine.
† Per cent radioactivity bound to TBG.
‡ Data of Solomon, D. H. (Los Angeles); personal communication.
§ Range of replicate values in sera of 6 normal controls by the same method (20).
¶ Concurrently analyzed sera.

serum PBI (8.9 μg. per 100 ml.) and for the thyroidal incorporation of I^{127} (230 and 167 μg. per day). Concentrations of BEI^{131} were not appreciably above normal. Thyroxine-binding capacities of TBG and pre-albumin did not deviate significantly from normal values. Four weeks after a course of treatment with methotrexate, the concentration of PBI had decreased to a value within the normal nonpregnant range (7.0 μg. per cent).

In Patient D.P., although there were no changes in the 24-hour thyroidal uptake of I^{131}, the calculated daily thyroidal incorporation of I^{127} was greater before treatment (110 and 132 μg.) than during subsequent study (44 and 54 μg.). The concentrations of PBI^{127} and BEI^{131}, however, were unchanged after surgery. The thyroxine-binding capacity of TBG was similar to that in the normal nonpregnant subject; however, the binding capacity of pre-albumin was reduced below that characteristic of normal pregnancy and the normal nonpregnant subject.

Molar pregnancy

Clinically, Patients P.G. and M.W. presented with signs of pre-eclamptic toxemia. Patient A.B., on the other hand, exhibited no such stigmata. Titers of chorionic gonadotropin were high in all 3 patients and were negative at the time of the second studies. Increased values for the 24-hour thyroidal uptake of I^{131} and the calculated daily incorporation of I^{127} were found in both of the patients in whom measurements were made. In one of these, P.G., values equalled those found in thyrotoxicosis. Thus, the 24-hour thyroidal uptake of I^{131} was 82 per cent of the administered dose, calculated thyroidal incorporation of I^{127} was 2,147 and 3,417 μg. daily, the concentration of serum PBI was 13.5 μg. per 100 ml., and the concentrations of serum BEI^{131} were 0.10 per cent of the administered dose per liter at both twenty-four and seventy-two hours. Six weeks after interruption of the pregnancy, values of these parameters had returned completely to normal. As in Patient P.G., the serum concentrations of PBI in Patient M.W. far exceeded the values expected during normal pregnancy. Even after hysterotomy and transfusion of 5 units of blood, the PBI level had decreased from the pretreatment value of 20.5 μg. per 100 ml. only to 11.9 μg. per 100 ml. Yet, six weeks later, without further treatment, the concentration of PBI and the 24-hour thyroidal I^{131} uptake were normal.

Changes in thyroidal economy observed in Patient A.B. were less striking. The initial concentration of serum PBI was 9.0 μg. per 100 ml. Although this value was in the hyperthyroid range, it was still within the range observed during normal pregnancy (3). The concentration of PBI subsequently decreased over an eight-day period to 7.2 μg. per 100 ml. Reduction to normal of the accumulation of I^{131} and I^{127} also occurred after treatment. A small decrease, of questionable significance, was observed

in the serum concentrations of BEI^{131} at twenty-four and seventy-two hours.

In contrast to the women with choriocarcinoma, the thyroxine-binding capacity of TBG was increased in the serum of all 3 women with hydatidiform mole. The saturation capacity of 150 μg. per 100 ml. noted in Patient P.B. exceeded the range of values found during normal pregnancy, whereas the saturation capacity of TBPA was greatly diminished.

DISCUSSION

Several recent attempts have been made to clarify further the origin and significance of the alterations in iodine metabolism characteristic of normal pregnancy. It has been found that administration of estrogen to nonpregnant subjects induces an increase in TBG and in PBI comparable to those observed during normal pregnancy (13, 14). As during the first half of pregnancy (15), these changes are unaccompanied by increases in basal oxygen consumption (16)—a paradox which could result from an antimetabolic effect of estrogen upon the tissues (17), altered binding of thyroxine within the circulation (18), or other phenomena.

Studies performed during administration of estrogen to nonpregnant subjects further indicate that the increase in the concentration of circulating thyroid hormone results from slowing of the rate of clearance of thyroxine from the circulation, which is of a degree sufficient to preserve unchanged the total daily quantity of hormone degraded (16, 19). Concomitantly, no evidence for increased thyroidal function during an estrogen-induced increase in the concentration of serum PBI has been found (20). Although a similar response to endogenous estrogen, as yet unproved, could account for the altered metabolism of iodine during pregnancy, several differences exist between this state and the circumstances of treatment of nonpregnant subjects with estrogen.

In contrast to the lack of effect of estrogen on thyroidal I^{131} accumulation in nonpregnant subjects, during pregnancy there is evidence for increased thyroidal avidity for iodide (2). Further, the alterations in metabolism of the pregnant woman are far more complex than those induced in the estrogen-treated subject. Thus, the reasons for the augmented thyroidal avidity for iodine, and the possibility that factors other than increased endogenous production of estrogen might participate in the changes in thyroxine-binding by TBG and the concentration of PBI during pregnancy, have required further exploration.

Among the several additional factors to be considered are the effects of 1) altered glucocorticoid metabolism, 2) increased elaboration of thyrotropin-like activity, and 3) elaboration of chorionic gonadotropin. Since the thyroidal effects of glucocorticoid in man are inhibitory (21), and

thyrotropic activity has not been demonstrable in pregnant hypophysectomized rats (22), the first two factors appear to be unlikely contributors to the changes in the metabolism of iodine during pregnancy. Failure to observe an increase in the saturation capacity of TBG in women with choriocarcinoma whose excretion of gonadotropin equalled that of normal pregnant women appears to exclude a role for this hormone in the induction of changes in the binding protein.

Parenthetically, this latter finding, and the failure to observe clinical signs of estrogenic effects in these women suggest that either the malignant trophoblast did not elaborate appreciable quantities of estrogen, or that the tropin detected in the urine lacked the capacity to stimulate intensely the ovarian or other sources of estrogen—an interpretation consistent with the concept that placental gonadotropin is predominantly luteotropic (23). The lack of estrogenic effects in these women is further in accord with the clinical observations that although men with testicular choriocarcinoma may excrete large quantities of both estrogen and gonadotropin (24), high gonadotropin excretion is commonly unaccompanied by high estrogen excretion in women with placental choriocarcinoma (25, 26).

From the limited data obtained in the study of the 2 women with choriocarcinoma there is evidence that thyroidal synthesis of hormone may differ before and after treatment of the malignant lesion. However, rates of turnover of the thyroid hormone, as judged by the concentrations of BEI^{131}, were not appreciably increased in either patient. Although the metabolism of iodine may have been influenced by the presence of choriocarcinoma, it is equally possible that the post-treatment results were influenced by therapy *per se*, since both patients were restudied following surgery and treatment with methotrexate, which is a profound inhibitor of folic-acid metabolism.

According to the present data, women with hydatidiform mole resemble normally pregnant women in the development of major alterations in thyroidal economy, but the changes appear to be augmented. Thus, one or more of the patients demonstrated 1) increased basal oxygen consumption beyond that expected during normal pregnancy of similar duration, 2) an extreme increase in the serum concentration of PBI beyond values observed during normal pregnancy, 3) evidence of greatly increased production of thyroid hormone as judged from increased thyroidal accumulation of I^{127}, and 4) an increase in the rate of turnover of thyroidal organic iodine, as shown by an increased concentration of circulating PBI^{131}, confirmed to be thyronine-I^{131} on the basis of the parallel increase in the concentration of BEI^{131}. In Patient P.G., the possibility exists that a part of the marked increase in thyroidal accumulation of iodide resulted from an acute reduction in the rate of glomerular filtration (27). However, the normal values

for the routine measures of renal function make this possibility unlikely.

In addition to the foregoing alterations in the metabolism of iodine, women with advanced hydatidiform mole exhibit cachexia, agitation, hyperkinetic circulatory dynamics, increased diaphoresis and increased skin temperature—stigmata which resemble those of Graves' disease. Theoretically, in the face of persistent stimuli toward increased hormonal synthesis, loss of those factors characteristic of normal pregnancy which protect the maternal organism against increased quantities of circulating hormone could induce a state of peripheral thyrotoxicosis. The finding in Patient A.B. of reduced thyroxine-binding by pre-albumin, as is also found in a variety of sick patients (28), is of interest in this regard.

In spite of clinical and laboratory data suggestive of thyrotoxicosis, none of the present patients demonstrated significant thyroidal enlargement or eye signs of thyrotoxicosis. In only one was there a bruit over the gland, and this disappeared after transfusions of blood. Furthermore, by the sole expedient of interrupting the pathologic pregnancy, the abnormal clinical and laboratory signs reverted to normal. What role, if any, the marked increase in thyroidal production of hormone may play in the pathogenesis of the clinical features of hydatidiform mole must await new knowledge of the physiologic significance of the altered thyroidal economy in this disease and in normal pregnancy. In view of the rarity of this pathologic pregnancy it is hoped that these few data will stimulate others to enlarge the pattern of observations whenever opportunities are presented.

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REFERENCES

1. Mussey, R. D.: Thyroid gland and pregnancy, *Am. J. Obst. & Gynec.* **36:** 529, 1938.
2. Halnan, K. E.: The radioiodine uptake of the human thyroid in pregnancy, *Clin. Sc.* **17:** 281, 1958.
3. Heinemann, M.; Johnson, C. E., and Man, E. B.: The serum precipitable iodine concentration during pregnancy, *J. Clin. Invest.* **27:** 91, 1948.
4. Ferraris, G. M., and Scorta, A.: Comportamento di alcuni tests di funzione tiroidea in gravidanza e in puerperio, *Minerva ginec.* **7:** 308, 1955.
5. Ingbar, S. H., and Freinkel, N.: Simultaneous estimation of rates of thyroxine degradation and thyroid hormone synthesis, *J. Clin. Invest.* **34:** 808, 1955.
6. Stanley, M. M.: The direct estimation of the rate of thyroid hormone formation in man. The effect of the iodide ion on the thyroid iodine utilization, *J. Clin. Endocrinol.* **9:** 941, 1949.

7. Burrows, B. A., and Ross, J. F.: The thyroidal uptake of stable iodine compared with the serum concentration of protein-bound iodine in normal subjects and in patients with thyroid disease, *J. Clin. Endocrinol. & Metab.* **13**: 1358, 1953.
8. Freedberg, A. S.; Ureles, A., and Hertz, S.: Serum level of protein bound radioactive iodine (I^{131}) in the diagnosis of hyperthyroidism, *Proc. Soc. Exper. Biol. & Med.* **70**: 679, 1949.
9. Ingbar, S. H.; Freinkel, N.; Hoeprich, P. D., and Athens, J. W.: The concentration and significance of butanol-extractable I^{131} of serum in patients with diverse states of thyroidal function, *J. Clin. Invest.* **33**: 388, 1954.
10. Freinkel, N.; Dowling, J. T., and Ingbar, S. H.: The interaction of thyroxine with plasma proteins: localization of thyroxine-binding protein in Cohn fractions of plasma, *J. Clin. Invest.* **34**: 1698, 1955.
11. Ingbar, S. H.: Pre-albumin: a thyroxine-binding protein of human plasma, *Endocrinology* **63**: 256, 1958.
12. Dowling, J. T., and Goodner, C. J.: Electronic analog integrator for simultaneous linear and integral graphic data recording. (Manuscript in preparation.)
13. Engstrom, W. W., and Markardt, B.: Influence of estrogen on thyroid function, *J. Clin. Endocrinol. & Metab.* **14**: 215, 1954.
14. Dowling, J. T.; Freinkel, N., and Ingbar, S. H.: Thyroxine-binding by sera of pregnant women, new-born infants, and women with spontaneous abortion, *J. Clin. Invest.* **35**: 1263, 1956.
15. Sandiford, I., and Wheeler, T.: The basal metabolism before, during and after pregnancy, *J. Biol. Chem.* **62**: 329, 1924.
16. Dowling, J. T.; Freinkel, N., and Ingbar, S. H.: The effect of estrogen in peripheral thyroxine degradation. Abstract No. 12 in Program of Annual Meeting of the American Goiter Association, San Francisco, California, June 17–19, 1958.
17. Guidry, M. A., Segaloff, A., and Altschul, A. M.: The effects of a-estradiol, some of its esters and estrone on the respiration of tissue homogenates, *Endocrinology* **50**: 29, 1952.
18. Dowling, J. T.; Freinkel, N., and Ingbar, S. H.: Effect of diethylstilbestrol on the binding of thyroxine in serum, *J. Clin. Endocrinol. & Metab.* **16**: 1491, 1956.
19. Engbring, N. H., and Engstrom, W. W.: Effects of estrogen and testosterone on circulating thyroid hormone, *J. Clin. Endocrinol. & Metab.* **19**: 783, 1959.
20. Dowling, J. T.; Ingbar, S. H., and Freinkel, N.: Failure of diethylstilbestrol to affect thyroidal accumulation and renal clearance of I^{131}, *J. Clin. Endocrinol. & Metab.* **19**: 1245, 1959.
21. Ingbar, S. H., and Freinkel, N.: ACTH, cortisone and the metabolism of iodine, *Metabolism* **5**: 652, 1956.
22. Greer, M. A.: Trophic hormones of the placenta: failure to demonstrate thyrotrophin or adrenocorticotrophin production in the hypophysectomized pregnant rat, *Endocrinology* **45**: 178, 1959.
23. Brown, W. E., and Bradbury, J. T.: A study of the physiologic action of human chorionic hormone. The production of pseudopregnancy in women by chorionic hormone, *Am. J. Obst. & Gynec.* **53**: 749, 1947.
24. Hamburger, C.: Gonadotrophins, androgens and oestrogens in cases of malignant tumours of the testis, *in* Hormone Production in Endocrine Tumours, ed. by G. E. W. Wolstenholme and M. O'Connor. Vol. XII of Ciba Foundation Colloquia on Endocrinology. Boston; Little Brown & Co., 1958, pp. 200–207.
25. Finkelstein, M.: Discussion of Hamburger, C., ref. 24. *Ibid.*, p. 213.

26. LORAINE, J. A.: Clinical Application of Hormone Assay. Edinburgh & London, E. S. Livingston Ltd., 1958, p. 198.
27. BRICKER, N. S., and HLAD, C. J., JR.: Observations on the mechanism of the renal clearance of I^{131}, *J. Clin. Invest.* **34**: 1057, 1955.
28. RICHARDS, J. B., DOWLING, J. T., and INGBAR, S. H.: Alterations in the plasma transport of thyroxine in sick patients and their relation to the abnormality in Graves' disease, (abstract) *J. Clin. Invest.* **38**: 1035, 1949.

THE EFFECT OF 2,3-DIMERCAPTOPROPANOL (BAL) ON THYROID FUNCTION*†

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ABSTRACT

During the clinical appraisal of a patient with acute arsenical poisoning, the suspicion of Graves' disease was entertained. An unexpected low thyroidal I^{131} uptake was observed. Since the patient was receiving BAL (British anti-lewisite) and since subsequent tracers confirmed the clinical diagnosis of Graves' disease, an investigation of the effect of BAL on thyroid function was undertaken. In a euthyroid male with arsenical poisoning, a similar inhibition of thyroidal I^{131} uptake was observed during treatment with BAL. Several series of rats (total of 116 experimental and control animals) were given varying doses of BAL ranging up to LD_{50}. A block in the thyroidal uptake of radioiodine was demonstrated, dependent upon the magnitude of the dose of BAL. By administration of BAL at three-hour intervals, a virtually complete block was effected. Removal of the thyroids and quantitative chromatographic separation of the iodinated compounds in the glands demonstrated that the radioactivity present was almost entirely in the iodide form.

OF THE numerous sulfhydryl-containing antithyroid drugs, the heterocyclic compounds have been shown to be more active than the straight-chain types (1). Clinical observations to be briefly described have indicated that one of the latter group, 2,3-dimercaptopropanol, more commonly referred to as British anti-lewisite (BAL), possesses considerable antithyroid activity.

It was a chance observation that drew special attention to this substance. A 40-year-old white woman who had ingested an arsenical preparation (Paris green) was being treated with a standard preparation of BAL.[1] Shortly after BAL therapy had been instituted, a possible diagnosis of Graves' disease was entertained, owing to the presence of a soft, diffusely enlarged thyroid, warm moist skin, a fine tremor of the outstretched

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[1] BAL 10% and benzyl benzoate 20% in peanut oil (100 mg./ml.), Hynson, Westcott & Dunning, Inc.—the only preparation of BAL available for study.

fingers, tachycardia, and a staring facial expression. In view of these clinical findings, a high uptake of radioiodine by the thyroid was anticipated. Contrary to expectations, a tracer dose of I^{131} given on the fourth day of BAL therapy (2.6 mg. of BAL per Kg. of body weight intramuscularly every six hours) resulted in a thyroidal I^{131} uptake of only 35 per cent in twenty-four hours. A reason for this discrepancy was sought.

The administration of BAL was continued for ten days, the frequency of administration being reduced to a single daily injection during the last three days of therapy. A second tracer dose of I^{131} given eight hours after the last injection of BAL (2.6 mg. per Kg.) resulted in an uptake of 64 per cent in twenty-four hours. One month following the initial arsenical poisoning, the I^{131} uptake was 69 per cent, and the serum protein-bound iodine (PBI) level was 5.6 μg. per 100 ml. It was suspected that either BAL or arsenic[2] accounted for the inhibition of I^{131} uptake. Experiments in animals were carried out to test these substances.

Subsequent to the following animal experiments, another patient with acute arsenical poisoning (sodium arsenite) was admitted to the hospital. Although this 26-year-old white male was not considered to have an abnormal thyroid, a tracer dose of I^{131} was given three hours after the first dose of BAL,[3] for comparison with another radioiodine study after completion of the BAL therapy. The initial I^{131} uptake was only 15 per cent in nineteen hours. Twenty-four hours following the discontinuation of BAL, the I^{131} uptake was 29 per cent in nineteen hours. This suggested that an antithyroid substance may also have been operative in this patient.

METHODS

A total of 116 white rats of the Sprague-Dawley strain, each weighing approximately 250 grams, were used. In order to insure a satisfactory uptake of radioiodine (I^{131}) by the thyroids of these animals, a diet containing an adequate, but not excessive, amount of iodine (.004 to .007 mg. of iodine per gram of food) was used for a week or more prior to the beginning of the experiments. In all of the later studies, the animals were pretreated with thiouracil (0.1 per cent in drinking water) for four days prior to the experiments in order to enhance the uptake of radioiodine by the thyroid. The thiouracil was discontinued twenty-four hours before the study was begun.

The same preparation of BAL which had been used in the clinical subjects was used in these experiments. It was usually administered subcutaneously at three-hour intervals. The LD_{50} for BAL, as previously reported (2), was 105 mg. per Kg. of body weight when administered intramuscularly. Preliminary experiments indicated that this

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[2] During the first twenty-four hours following ingestion of the arsenic, 7.4 mg. was excreted; in the second twenty-four hours, 4.9 mg.; and in the third twenty-four hours, 4.4 mg.

[3] Doses of 4.9 mg. and 2.5 mg. of BAL per Kg. of body weight were given three hours and one hour respectively before the I^{131} was given. The smaller dose was repeated at four-hour intervals but was discontinued eight hours before the radioactivity in the thyroid was counted.

value held for subcutaneous administration as well. Control experiments using only the vehicle for BAL—benzyl benzoate, 20 per cent in peanut oil—produced no observable effect.

The experiments to be described were designed to explore the blocking effect of BAL on the uptake of radioiodine by the thyroid. The magnitude of the effective dose and the promptness, duration and nature of the action were considered. A tracer dose of I^{131} (5, 8 or 10 microcuries) was injected intraperitoneally one hour after BAL had been given. A shielded scintillation counter with a small aperture to permit counting over the area of the thyroid was used at frequent intervals to follow the *in vivo* accumulation of I^{131}. When the animals were sacrificed, the thyroids were carefully dissected from the carcasses and their I^{131} content measured. In some experiments, the excised thyroids were subjected to hydrolysis at 100° C. in individual sealed tubes to which had been added 1 ml. of 4 *N* sodium hydroxide and a crystal of sodium thiosulphate. The digest was extracted and chromatographed, using a single dimension butanol:dioxane:ammonia solvent system as previously described (3).

RESULTS

The results confirmed the clinical observations that BAL interfered with the normal accumulation of iodine by the thyroid. In experiments in which I^{131} was given one hour after the administration of BAL, the thyroidal uptake of this isotope was impaired. Figure 1 shows curves representing the accumulation of I^{131} in a group of 4 control animals and 4 animals which had been given injections of 75 mg. of BAL per Kg. of body weight at three-hour intervals. The block to the uptake of I^{131} was effectively maintained throughout the experiment, yet the animals showed little evidence of toxicity.

In order to test the duration of the block produced by this amount of BAL, a single dose was given and the results were observed over a period of nine hours. As shown in the experiment illustrated in Figure 2*a*, the block was effectively maintained for nearly five hours. Thereafter the uptake proceeded at approximately the same rate as in the control animals. (Compare Figure 2*a* with Figure 1, showing the result of the same dose of BAL repeated at three-hour intervals.)

In Figure 2*b*, another group of rats given injections of 75 mg. of BAL per Kg. of body weight are compared with controls. A rise in the thyroidal uptake of I^{131} by the BAL animals was first noticed between three and four hours after the injection of BAL. Although the uptake of I^{131} had begun, an additional dose of BAL immediately arrested any further rise in the curve.

Experiments were performed using doses of 25, 50, 75 and 100 mg. of BAL per Kg. of body weight, to demonstrate the primary effectiveness of the block and to ascertain the duration of the effect. Although considerable individual variation in the initial effectiveness and duration of the block was observed, an effective block could be demonstrated in all animals receiving 50 mg. of BAL per Kg. of body weight or more. In Figure 3

it may be seen that the responses of the animals were relatively uniform. In this experiment, one group of animals received 25 mg. of BAL per Kg. of body weight and another, 75 mg. per Kg. The difference in effect between these two doses is clearly demonstrated in Figure 3.

The dose of 25 mg. of BAL per Kg. of body weight was in no case sufficient to produce a block that would distinguish the I^{131} uptake of this group from that of the controls. However, this dose presumably had a subminimal effect, since a second dose of 25 mg. of BAL per Kg. (superimposed three hours after the first) produced a block in all animals, even though it was of relatively short duration. The thyroidal accumulation of I^{131} was resumed at the same rate as in the control group in approximately two hours. These experiments indicate that BAL has a rather short period of action, the duration of the block being related to the size of the dose. When the period of effective block begins to wane, the thyroidal uptake of radioiodine follows the course of uptake observed in the controls. If BAL is again administered, further uptake of radioiodine is arrested within a few minutes.

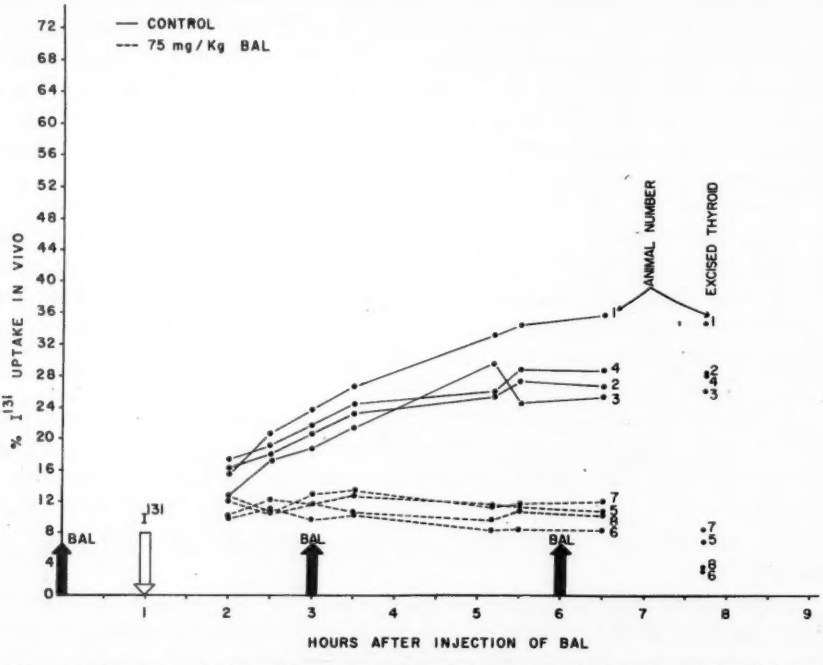
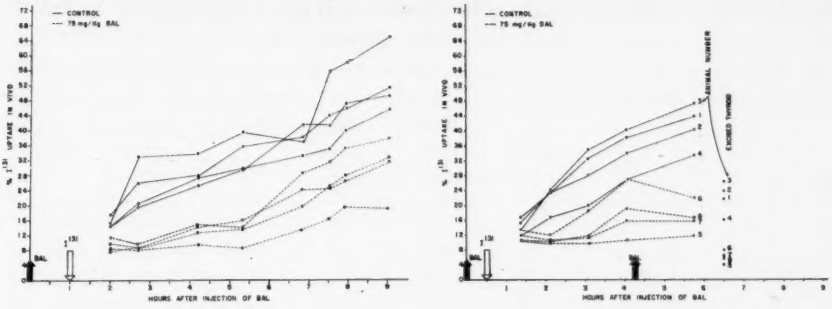


FIG. 1. The accumulation of I^{131} by the thyroid in rats receiving 75 mg. of BAL/Kg. of body weight every three hours, as compared with controls.

The I^{131} determinations on the extreme right were carried out on the excised gland following the sacrifice of the animal. The accompanying numbers identify the animals for comparison with the *in vivo* uptake to the left.



a b

FIG. 2. **a.** The accumulation of I^{131} by the thyroid in rats receving a single injection of 75 mg. of BAL/Kg. of body weight, as compared with controls. In this group the uptake of I^{131} by the thyroid began shortly after five hours following the injection of BAL. The rate of accumulation after that time resembled that in the controls.

b. The accumulation of I^{131} by the thyroid in rats receiving 75 mg. of BAL/Kg. of body weight as compared with controls. A second dose of 75 mg. per Kg. was given four and a quarter hours after the first. This caused prompt interruption of the accumulation which had begun as the effect of the first dose was diminishing.

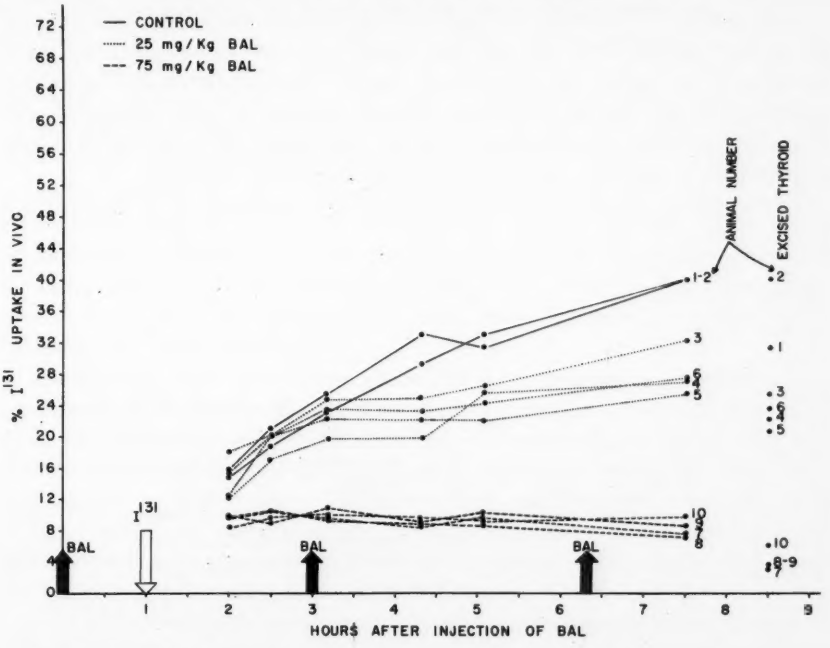


FIG. 3. I^{131} uptake, showing the effect of varying doses of BAL.

A dose of 75 mg. of BAL/Kg. of body weight produced a block, whereas a dose of 25 mg. per Kg. did not. The administration of a second dose of 25 mg. per Kg. three hours later augmented the first, and thus produced a block of short duration.

TABLE 1. THE RESULTS OF CHROMATOGRAMS PREPARED FROM RAT THYROIDS UNDER THE INFLUENCE OF BAL.

(The uptake of I^{131} by the thyroids described here can be seen in Figure 2b.)

Group	Rat No.	Monoiodotyrosine	Diiodotyrosine	Triiodothyronine	Thyroxine	Total organic iodine	Iodide
Controls	1	14	19	0	1	34	66
	2	21	27	1	2	51	49
	3	26	28	3	14	71	29
	4	36	17	3	6	62	38
BAL 75 mg./Kg.	5	30	1	0	0	31	69
	6	10	8	1	3	22	78
	7	20	9	1	4	34	66
	8	8	7	4	5	24	76

The quantitative chromatograms prepared from the digests of the thyroid glands revealed that radioactivity in the thyroids which had been under the influence of large doses of BAL was predominantly in the form of free iodide. In contrast, in the control animals there was much more thyroidal radioactivity, and the chromatograms contained relatively large amounts of I^{131} in the organic forms. Table 1 shows the relative percentages of radioactivity in iodide and organic forms in the two groups of rats. Representative chromatograms of the thyroid-digest of an animal that had received BAL and of a control animal are shown in Figure 4.

Experiments to test the effect of acute doses of arsenic (2.5, 5.0 and 10.0

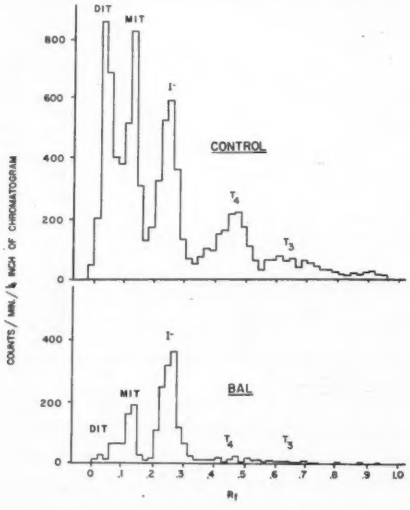


FIG. 4. Chromatograms of digest of the thyroids of a control rat and a rat which had received 75 mg. of BAL/Kg. of body weight.

MIT = monoiodotyrosine; DIT = diiodotyrosine; T_4 = thyroxine; T_3 = triiodothyronine; I = iodide.

mg. of arsenic trioxide per Kg. of body weight) in rats were carried out in a manner similar to that described for BAL. Only occasionally was there impairment of the thyroidal uptake of I^{131}. When some inhibition did occur, there was no correlation between the dose of arsenic and the wide range of I^{131} uptake observed.

DISCUSSION

When these experiments were initiated, it was felt that either BAL or arsenic interfered with the incorporation of I^{131} into the thyroid. It had been suggested by Scott (4) that arsenic may act as a goitrogen, on the grounds that goiter was found to be prevalent in geographical areas rich in arsenic. Proof of this relationship was sought in experiments reported by Sharpless and Metzger (5). They found that when arsenic pentoxide was added to the basal diet for several weeks in quantities of 20 mg. per 100 Gm. of food, there was a significant decrease in the iodine content of the thyroid and an increase in the weight of the gland. Smaller doses, of the order of 5 mg. per 100 Gm. of food, failed to produce such changes.

Förster and Muscholl (6), while studying the effect of BAL on the hyperplasia of the thyroid produced by methylthiouracil, found that BAL did not affect the metabolism of rats when given in doses of 30 mg. per Kg. of body weight (frequency of dose not recorded). In control studies in which they described the administration of BAL alone, the thyroid showed marked histologic evidence of activation without an increase in the weight of the gland. In further experiments on rats to test the influence of BAL and methionine on the effect of iodine on the thyroid, Förster *et al.* (7) recorded evidence which suggests that BAL given alone inhibits the function of the gland. For example, in the animals receiving BAL there was a marked gain in body weight, and the metabolism, as measured by oxygen consumption, was below that in the normal rats. Furthermore, histologic study of the anterior pituitary revealed changes which resembled those produced by thyroidectomy.

The demonstrated antithyroid effect of BAL prompted an inquiry into the chemical similarity of this substance to other known goitrogens. The most potent antithyroid substances fall into two categories, *viz*, thiourea and related substances, and derivatives of aniline (1). Although BAL does not possess a double-bonded sulfur radical on a carbon adjacent to a nitrogen atom, an arrangement which is prominent in the more active antithyroid drugs, the presence of two sulfhydryl groups probably accounts for the goitrogenic properties of this substance.

It is difficult to determine from these experiments whether the action of BAL represents a competitive substance or a blocking effect on enzyme, as with thiocarbamides. Chromatography performed on thyroids which were apparently completely blocked with BAL at the time of the last *in vivo*

observation revealed the presence of some iodinated organic compounds. It is unlikely that the thyroidal uptake of I^{131} and organic binding of it occurred between the last *in vivo* measurement and the time of sacrifice of the animal, because the last dose of BAL was so arranged as to cover this interval of time. It is possible that some synthesis may have occurred after the gland was excised and before hydrolysis was accomplished. Enzyme systems involved in the organic binding of iodine may have survived longer than BAL in the excised tissue and may have converted some trapped iodide to the organic forms. The synthesis of organic iodinated compounds was inhibited during hydrolysis by the addition of sodium thiosulfate, which in previous control experiments (8) has been shown to block any further formation of iodinated organic compounds during the process of hydrolysis and subsequent chromatography.

In the studies described here, BAL was effective in blocking the uptake of I^{131} by the thyroid in both man and rats. It is well known that some substances which are highly potent antithyroid drugs in man are of low potency in rats, and vice versa (9). Since this wide species variation in the response to antithyroid drugs does exist, it is not surprising that the effective dose of BAL in the rat was 10 to 20 times that in man in these studies.

REFERENCES

1. Astwood, E. B.: Mechanism of action of antithyroid compounds, *Brookhaven Symposia in Biology* No. 7, pp. 61–73 (Feb.) 1955.
2. Waters, L. L., and Stock, C.: BAL (British anti-lewisite), *Science* **102:** 601 (Dec. 14) 1945.
3. Benua, R. S.; Dobyns, B. M., and Ninmer, A.: Triiodothyronine in the serum of patients treated with radioactive iodine, *J. Clin. Endocrinol. & Metab.* **15:** 1367–1378 (Nov.) 1955.
4. Scott, M.: The possible role of arsenic in the etiology of goiter, cretinism, and endemic deaf-mutism, *Tr. 3rd Internat. Goiter Conference and Am. A. Study Goiter* pp. 34–49, 1938.
5. Sharpless, G. R., and Metzger, M.: Arsenic and goiter, *J. Nutrition* **21:** 341–346 (Apr.) 1941.
6. Förster, W., and Muscholl, E.: Der Einfluss von BAL und Methionin auf die Schilddrüsenhyperplasie nach chronischer Gabe von Methylthiouracil, *Arch. exper. Path. u. Pharmakol* **222:** 408–412, 1954.
7. Föster, W.; Herrmann, C.; Scharf, J. H., and Ehrenbrand, F.: Korrelationen zwischen Stoffwechsel-, Schilddrüsen- und Hypophysenveränderungen bei Ratten nach chronischer Verabreichung von NaJ, BAL und Methionin, *Arch. exper. Path. u. Pharmakol.* **225:** 195–209, 1955.
8. Dobyns, B. M., and Barry, S. R.: The isolation of iodinated amino acids from thyroid tissue by means of starch column chromatography, *J. Biol. Chem.* **204:** 517–531 (Sept.) 1953.
9. Astwood, E. B., and Solomon, D. H.: Mechanisms of action of antithyroid drugs, iodides, and other thyroid inhibitors, *in* The Thyroid: A Fundamental and Clinical Text, ed. by S. C. Werner. New York, Paul B. Hoeber, Inc., 1955, Chapt 6, pp. 48–60.

STUDIES ON PROTEINS OF NORMAL AND DISEASED THYROID GLANDS*†

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ABSTRACT

Distribution of protein and I^{131} in centrifugally fractionated thyroid homogenates, and the electrophoretic, solubility and ultracentrifuge characteristics of the soluble fraction were studied in normal and diseased human thyroid glands. In the normal glands, 64–82 per cent of the protein and 95 per cent of the I^{131}-label appeared in the soluble fraction. Thyroglobulin comprised 70–79 per cent of the soluble protein. Normal thyroglobulin had a Svedberg constant (0.5 per cent solution in 0.9 per cent NaCl) of 18–20, and salted out of phosphate buffer at 1.7–1.9 *M*. No protein with greater mobility than thyroglobulin was detected on paper electrophoresis in barbital buffer. In all diseased glands, an increased proportion of light-weight ($S_{w,20}=4$) protein was observed, and the thyroglobulin usually had greater solubility in phosphate buffer. In the soluble fraction of some colloid goiters, 1 follicular adenoma, 3 congenital goiters and 1 thyroid carcinoma, a protein as yet indistinguishable from albumin was detected. An abnormal iodinated soluble protein was detected in 1 congenital goiter, in which gland thyroglobulin was absent. Colloid goiters were associated with an increased proportion of soluble protein and I^{131}, whereas congenital goiter and carcinoma were associated with an increased proportion of particulate protein and I^{131}. The alterations observed are apparently due to 1) an accumulation of albumin-like protein in colloid goiters, some follicular adenomas and some congenital goiters, and 2) a deficient production of thyroglobulin in some congenital goiters and in thyroid carcinoma. It is suggested that abnormalities in the metabolism of proteins other than thyroglobulin could play a role in the production of nodular colloid goiter.

INFORMATION on the proteins of the thyroid gland in health and disease has gradually accumulated in recent years. The protein of principal interest, thyroglobulin, has been characterized as a glycoprotein (1) of molecular weight 650,000 (2), with a sedimentation constant of $S_{w,20}=19$ (3). The iodine content (4), ratio of iodotyrosines to iodothyronines (5), ratio of monoiodotyrosine (MIT) to diiodotyrosine (DIT) (6), solubility in salt solutions (7), electrophoretic mobility (8) and perhaps antigenic characteristics (9) are variable.

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‡ Fellow of the National Foundation.

Other iodinated proteins, presumably of thyroidal origin and smaller molecular size, have been detected in the serum of patients with thyroid carcinoma (10), hyperthyroidism (11, 12), and congenital goiter (13, 14). More recently, similar iodinated proteins apparently distinct from thyroglobulin were found in normal human thyroids (15), adenomas, nodular goiters (15) and congenital goiter (16), as well as in rat thyroid tumors (17) and normal sheep thyroid glands (15). An abnormal attachment of protein to animal and human thyroid subcellular particles has also been inferred (15). Lastly, an iodinated protein distinct from thyroglobulin has been solubilized from its attachment to sheep or human thyroid mitochondria and microsomes by the action of desoxycholate (18). No evidence has been presented that any of these proteins is a precursor or degradation product of thyroglobulin.

The present project was undertaken as an extension of studies of an abnormal iodinated protein detected in the serum and thyroid of certain patients with congenital goiter (13). It seemed possible that defective synthesis or degradation of thyroglobulin might play a primary role in the pathogenesis of some of the thyroid disease states, rather than defective iodide metabolism *per se*. Accordingly, an attempt was made to characterize the iodinated and noniodinated proteins of the thyroid gland. Concurrently, data were accumulated on the distribution of I^{131} and protein among the subcellular fractions of the gland, since it seemed possible that defective synthesis of thyroglobulin might be reflected by an accumulation of normal iodinated protein in one of these fractions.

Some of the data on congenital goiters and normal tissue have been presented in detail elsewhere (16) but are included here for comparison.

METHODS

Specimens of human thyroid tissue which were labeled by administration of I^{131} twenty-four to two hundred hours previously, were obtained at thyroidectomy or open biopsy and immediately cooled to 4° C. Representative portions free of cysts or hemorrhage were excised, rinsed with saline, and homogenized with four times their weight of .14 *M* sodium chloride or 0.25 *M* sucrose. Aliquots of homogenate were spun in an International refrigerated centrifuge (Model PR 2) at 700 times gravity (×g) for ten minutes at 0° C. The supernatant was removed. The resuspended sediment was centrifuged again at 700×g for ten minutes. This process was repeated again, and the final sediment was made up to 50 per cent of the original volume. The combined supernatant fractions were centrifuged at 8500×g for twenty minutes at 0° C. in the angle high-speed head of the centrifuge and washed once under the same conditions. The sediment was made up to 50 per cent of the original volume. The combined 8500×g supernatant fraction was spun at 105,000×g for one hour at 0° C. in the Spinco preparative ultracentrifuge using the No. 40 rotor. After centrifugation the supernatant fluid was removed by a pipette, and the tube was rinsed by carefully adding 3 ml. of fluid, twirling the tube, and pouring off the rinse fluid, which was discarded. The sediment was made up to 50 per cent of the original volume.

Samples homogenized in .14 *M* sodium chloride were carried through the entire procedure using sodium chloride, or else were homogenized and carried through using .25 *M* sucrose. Preparations made in .14 *M* sodium chloride had more of a tendency to clump than those prepared in 0.25 *M* sucrose. The 700×g and 8500×g sediments probably would be more heavily contaminated with lighter weight particles in preparations using saline. Quarter molar sucrose was used rather than an isosmotic solution, since this concentration is widely employed to achieve maximum oxidative phosphorylation rates in homogenate fractions. Calcium was not added.

In one instance, thyroid gland slices were labeled *in vitro* by incubation in Krebs Ringer phosphate buffer with I^{131} for two hours at 37° C. The slices were rinsed after incubation, and then treated as were *in vivo* labeled specimens.

The 700×g precipitate was microscopically a mixture of nuclei and cell debris. The 8500×g fraction appeared microscopically to be made up of uniformly granular material and rare nuclei, but was considered to be a mixture of mitochondria and microsomes. The 105,000×g sediment was thought to represent microsomes. The 105,000×g supernatant was similar to a saline or sucrose extract of the gland and contained the soluble proteins.

The *supernatant fraction* was studied by electrophoresis on Whatman No. 1 paper at 70 volts, 5 amperes, for fourteen hours in pH 8.6 barbital-citrate .1 *M* buffer, by salting out in pH 6.8 phosphate buffer, by ultracentrifugation in the Spinco ultracentrifuge, and by immunologic techniques. For salting out of thyroidal proteins, a modification of the method of Robbins (19) was followed. One-tenth ml. of thyroid supernatant was added to 2 ml. of a mixture of distilled water and 3.5 *M* pH 6.8 phosphate buffer (in varying proportions) to yield a final concentration of .85–3.34 *M* phosphate. After forty hours at 4° C., the samples were shaken with ether, and spun at 700×g for ten minutes. Aliquots of the water layer were removed for determination of I^{131} and protein content. Results by this method gave a salting-out point for normal thyroglobulin of 1.7–1.9 *M*, which is somewhat higher than that reported by Robbins. This probably was an effect of salting out at 4° C., rather than 20°. It was observed that when specimens were incubated at 20° C. for twenty-four hours, samples in the most critical zone (1.7–2.0 *M*) remained cloudy after ether flocculation and centrifugation. This cloudiness presumably represented denatured but uncoagulated thyroglobulin, and its presence would tend to increase the apparent solubility of the thyroglobulin. This phenomenon was less apparent when samples were "incubated" and centrifuged at 4° C.

In some instances aliquots of the homogenate and each fraction were taken for I^{131} determination by counting in a well scintillation crystal, and for protein determination (20).

Five normal thyroid specimens, 6 colloid goiters, 4 adenomas, 4 congenital goiters, and 1 carcinoma were studied. Two discrete and morphologically distinct adenomas, and normal tissue, were taken from one specimen. Noncancerous tissue in the carcinoma specimen was also studied separately, and the data are included for comparison. The term colloid goiter as used in this presentation refers to the entity known variously as "sporadic colloid goiter," "nontoxic nodular goiter" or "adenomatous goiter."

RESULTS

I. Electrophoresis (Table 1, Fig. 1)

After electrophoresis of the normal thyroid tissue supernatant fraction, the major protein band appeared in an area analogous to that of serum

TABLE 1. PAPER ELECTROPHORESIS OF THYROID-HOMOGENATE SOLUBLE FRACTION

Category	Patient	Protein component		
		Prethyro-globulin	Thyro-globulin	Beta-Gamma zone
Normal	Sch	—	present	present
	Rot	—	present	present
	Zaz	—	present	present
Colloid goiter	Whi	—	present	present
	DeG	present	present	present
	Gav	present	present	present
	Bet	—	present	present
	Sir	—	present	present
	Qua	—	present	present
Adenoma, follicular	Pec	—	present	present
	Wal	present	present	present
	Rot	—	present	present
fetal	Rot	—	present	present
Congenital goiter	Cob	present	—	present
	For	present	present	present
	Chi	present	present	present
	Cle	—	present	present
Thyroid carcinoma	Val	present	present	present
Non-cancerous tissue	Val	—	present	present

α_1–α_2 globulins, as has been reported by others (21). A second more diffuse zone ("β-γ band") occupied the area of serum $\beta+\gamma$ globulin. In the normal gland, 90–95 per cent of the I^{131} was in the thyroglobulin zone.

In 2 of 6 specimens from colloid goiters, a distinct pre-thyroglobulin protein band was observed, but no I^{131} was associated with this zone. A similar band appeared in the preparations from 1 follicular adenoma, 3 of 4 cases of congenital goiter, and in 1 thyroid carcinoma. In only 1 instance, a case of congenital goiter in which thyroglobulin was absent, did the pre-thyroglobulin zone contain I^{131}. Within the limits of paper electrophoretic techniques, all of the thyroglobulins had the same mobility. Glycoproteins were detected by staining reactions only in the thyroglobulin zone.

II. Salting out of supernatant (Table 2, Fig. 2)

The I^{131}-labeled soluble protein of normal glands salted out of solution sharply between 1.7 and 1.9 *M* phosphate buffer. A portion of the iodinated protein remained soluble at 2 *M* (5–17 per cent), and less at 3 *M* (2–12 per cent).

In the diseased glands the major I^{131}-containing component became insoluble at higher phosphate concentrations, except in the case of 1 follicular

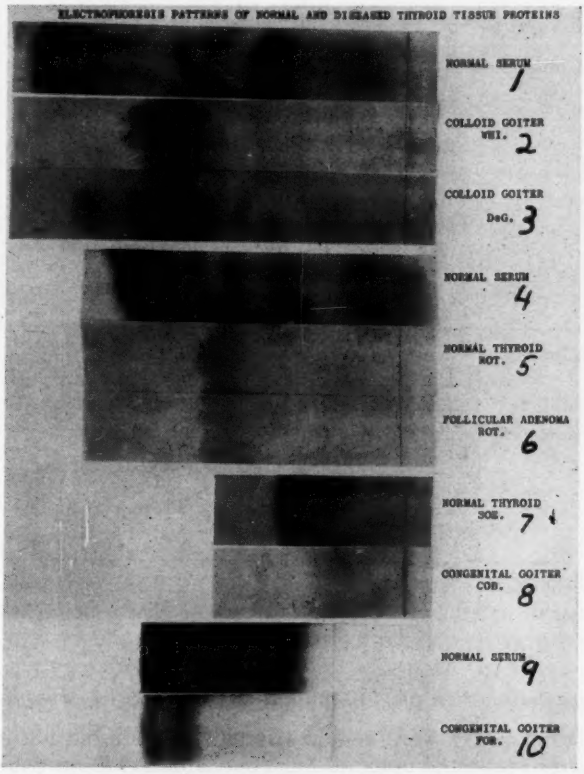

FIG. 1. Electrophoresis patterns of normal serum and of human thyroid gland soluble protein, in barbital citrate pH 8.6 buffer. **1.** Serum control for 2 and 3. **2.** Colloid goiter. **3.** Colloid goiter, demonstrating the pre-thyroglobulin noniodinated component. **4.** Normal serum control for 5 and 6. **5.** Normal thyroid proteins after 12-hour run. **6.** Follicular adenoma. **7.** Normal thyroid proteins after 7-hour run. **8.** Congenital goiter, demonstrating absence of thyroglobulin and presence of an iodinated prethyroglobulin band. **9.** Serum control for 10. **10.** Congenital goiter, demonstrating a striking pre-thyroglobulin component.

adenoma, and 1 gland classified tentatively as congenital goiter. In some instances no appreciable precipitation occurred until 2 *M* concentration was reached. There was also a tendency for salting out to occur in a less sharply defined zone over the range of 1.8–2.3 *M* buffer.

TABLE 2. SALTING OUT OF *in vivo*-LABELED THYROGLOBULINS IN pH 6.8 PHOSPHATE BUFFER

Category	Patient	Zone of precipitation, molarity	Method of determination	% I^{131} soluble	
				at 2*M*	at 3*M*
Normal	Sch	1.8–1.9	RAI & Protein	17	12
	Soe	1.7–1.9	RAI		
	Rot	1.7–1.83	RAI & Protein	5	2
Colloid goiter	Whi	1.7–2.0	RAI	16	2
	DeG	2.0–2.3	RAI	62	7
	Gav	1.7–2.0	Protein		
	Bet	1.8–2.6	Protein		
	Qua	1.75–2.3	RAI	37	3
Adenoma, follicular	Wal	1.8–2.2	Protein		
	Rot	1.7–1.83	RAI & Protein	12	6
Adenoma, fetal	Rot	1.7–2.0	RAI	10	0
Congenital goiter	Cob	1.4–2.5	RAI & Protein	79	3
	For	2.0–2.2	RAI	81	11
	Chi	1.8–2.6	Protein		
	Cle	1.7–1.9	RAI	5	1
Thyroid carcinoma	Val	1.8–2.3	RAI	64	2
Noncancerous tissue	Val	1.6–3.0	RAI	31	5

III. Ultracentrifugation of supernatant fraction in .14 M sodium chloride

Three distinct peaks were visible after ultracentrifugation in each normal supernatant fraction. The designations employed are taken from the study of Shulman *et al.* on pig thyroid extracts (22). Half per cent protein solutions were routinely examined. In some instances multiple concentrations were studied in order that the sedimentation constant could be obtained by extrapolation to zero concentration. Peak I, $S_{w,20}=28$, contained 3–7 per cent of protein; Peak II, $S_{w,20}=19$, contained 70–79 per cent of protein; and Peak V, $S_{w,20}=4.2$, contained 12–24 per cent of protein. In 1 specimen a small peak with $S_{w,20}=12$, containing 4 per cent of protein

(possibly Peak III of Shulman) was detected. Peak II is thyroglobulin. Peaks I and V have not been characterized, except that albumin and hemoglobin may appear in Peak V (22). Peak IV of Shulman, which was a minor component in the porcine gland, was either absent or too small to be detected in this study (Table 3, Fig. 3).

In the patterns for abnormal tissues, the mobility of the Svedberg peaks was essentially the same as for the normal gland. The variation observed was attributed to unavoidable error in the technique, and the effect of varying proportions of protein components on their respective mo-

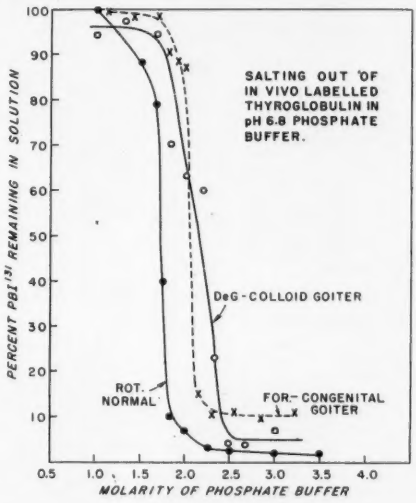

FIG. 2. Representative salting-out curves for 3 thyroid homogenate supernatant fractions in phosphate buffer.

bilities. The proportion of protein in each component varied markedly, with a tendency towards an increased proportion of Peak V, and a decreased proportion of Peak II (thyroglobulin). In the patterns for 4 colloid goiters Peak I was 2–7 per cent of protein, Peak II 56–74 per cent, and Peak V 23–42 per cent. Peak V was increased for 1 follicular adenoma and 1 fetal adenoma. In 4 cases of congenital goiter the proportion of Peak V was greatly increased, being 80 per cent of total protein in one instance, and 100 per cent in another. A similar distribution obtained for 1 thyroid carcinoma.

By use of the ultracentrifuge data, it was possible to determine the quality of thyroglobulin and $S_{w,20}=4$ proteins present per gram of thyroid tissue. It should be noted that the $S_{w,20}=4$ protein in Table 4 includes the normal $\beta+\gamma$ and, if present, the abnormal pre-thyroglobulin band visualized by paper electrophoresis.

TABLE 3. ULTRACENTRIFUGE ANALYSIS OF SUPERNATANT FRACTION PROTEINS: OBSERVED $S_{w,20}$ CONSTANTS AND PER CENT DISTRIBUTION OF TOTAL PROTEIN IN EACH COMPONENT

Category	Patient	I		II		III		V	
		Svedberg constant	% of protein	Svedberg constant	% of protein	Svedberg constant	% of protein	Svedberg constant	% of protein
Normal	Sch	28	6	19	70	—	—	4.1	24
	Soe	29	6	20	77	—	—	4.3	17
	Rot	27	7	18	77	12	4	4.6	12
	But	31	3	20	79	—	—	4.3	18
Colloid goiter	Whi	25	5	17	69	—	—	4.2	26
	DeG	27	2	18	56	—	—	4.3	42
	Gav	27	7	19	63	—	—	4.3	28
	Bet	30	3	19	74	—	—	4.8	23
Adenoma, follicular	Wal	30	8	21	64	—	—	4.3	28
	Rot	25	6	18	54	12	2	4.3	18
Adenoma, fetal	Rot	27	6	18	67	—	—	4.5	27
Congenital goiter	Cob	—	—	—	—	—	—	4.1	100
	For	—	—	20	51	—	—	4.5	49
	Chi	—	—	19	20	—	—	4.4	80
	Cle	28	7	18	72	—	—	4.3	21
Thyroid carcinoma	Val	—	—	20	26	—	—	4.5	74
Noncancerous tissue	Val	30	3	19	60	—	—	4.3	37

IV. Fractionation of thyroidal homogenate protein

In homogenates of 2 normal glands, 12–20 per cent of the total protein appeared in the nuclear fraction, 1.4–7.2 per cent in the mitochondrial fraction, 6.7–8.5 per cent in the microsomal fraction, and 64–82 per cent in the supernatant. This was similar to the results of fractionation of normal sheep tissue (nuclear fraction = 7.2 per cent, mitochondrial = 4.6 per cent, microsomal = 7 per cent, supernatant = 78 per cent). In the homogenate of 1 colloid goiter, 11 per cent of the protein was in the particulate fraction, and 89 per cent in the soluble portion. In the follicular adenomas, distribution was similar to that in the normal gland, but in the fetal adenoma a large proportion of the protein was in the particulate fraction. In the

homogenates of 2 congenital goiters there was an increase in proportion of particulate protein, associated in one instance with a marked decrease in thyroglobulin content. The findings were similar in the thyroid carcinoma.

V. Fractionation of thyroidal homogenate I^{131}

In the normal gland (2 cases), 0.7–3.9 per cent of the protein appeared in the nuclear fraction, 0.1–1.2 per cent in the mitochondrial, 0.7–1.7 per cent in the microsomal, and 95 per cent in the supernatant. In colloid

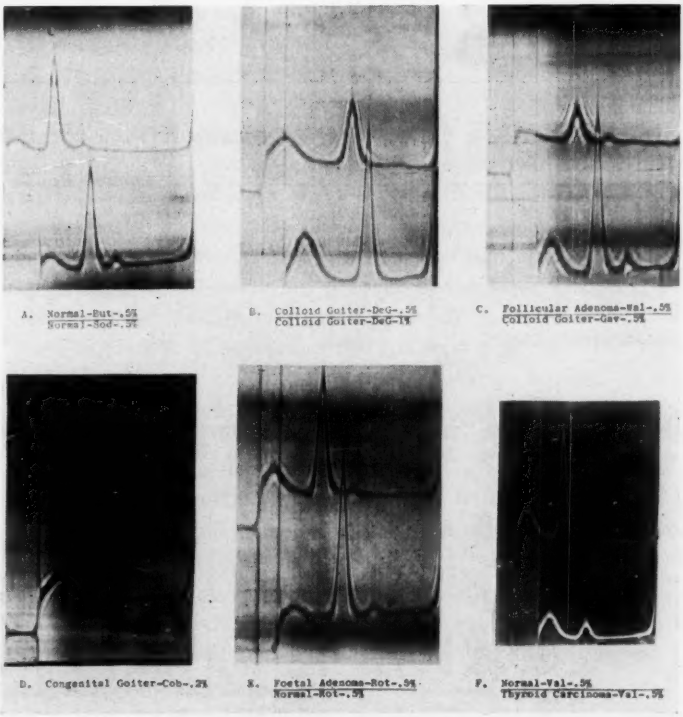

FIG. 3. Ultracentrifuge pattern of thyroid homogenate supernatant fraction proteins in .14 *M* NaCl. Direction of migration is to right. Duration of spin, approximately 55 minutes. All specimens, 0.5 per cent protein unless otherwise noted. **A,** upper—normal pattern showing components I, II (major peak, thyroglobulin) and V. **A,** lower—normal pattern. **B,** upper—colloid goiter showing increased proportion of component V. **B,** lower—same specimen in 1 per cent solution. **C,** upper—follicular adenoma with some increase in component V. **C,** lower—colloid goiter with moderate increase in component V. **D**—congenital goiter with only component V visible. **E,** upper—fetal adenoma with some increase in component V in comparison to normal specimen below. **F,** lower—thyroid tumor with no peak I, decrease in II, and increase in V, compared to noncancerous tissue from the same gland in **F,** upper.

TABLE 4. SOLUBLE PROTEIN OF THYROID TISSUE
(mg. protein per Gm. tissue (wet weight))

Category	Patient	Thyroglobulin	$S_{w,20}=4$ component
Normal	Soe	62	13
	Rot	102	16
	But	79	18
Colloid goiter	Whi	96	26
	DeG	50	38
	Gav	51	23
Adenoma, follicular	Rot	101	25
Adenoma, fetal	Rot	84	33
Congenital goiter	For	76	73
	Chi	11	42
	Cle	90	26
Thyroid carcinoma	Val	4	11
Noncancerous tissue	Val	32	20

TABLE 5. DISTRIBUTION OF TOTAL HOMOGENATE I^{131} AND PROTEIN IN CELL FRACTIONS

Category	Patient	Protein content, (% in fraction)				RAI content (% in fraction)			
		700 ×g	8,500 ×g	105,000 ×g	Super-nat.	700 ×g	8,500 ×g	105,000 ×g	Super-nat.
Normal	Sch					0.7	1.2	0.7	95.0
	Rot	12.1	1.4	6.3	81.5	3.9	0.1	1.7	94.5
	Sev	20.0	7.2	8.5	64.3				
Colloid goiter	Sir	3.3	3.7	4.2	88.8	0.6	0.6	2.0	96.8
Adenoma, follicular	Rot	11.6	2.3	6.4	79.5	3.6	0.1	1.9	94.2
Adenoma, fetal	Rot	18.2	4.0	7.0	71.2	2.4	0.3	1.3	96.5
Congenital goiter	Cob					15.0	8.0	5.0	72.0
	For					0.3	0.01	0.05	99.6
	Chi	(	46.0	)	54.0	(	10.3	)	89.7
	Cle	17.5	10.0	5.5	62.2	3.2	1.0	2.3	93.5
Thyroid carcinoma	Val	45.0	1.5	1.8	51.0	14.0	0.7	10.0	75.0
Noncancerous tissue	Val	8.3	12.8	15.0	64.0	5.5	1.9	1.3	91.0

goiter there was a greater proportion in the soluble fraction. I^{131} distribution in the adenomas was similar to that in the normal glands. In 2 congenital goiters and 1 thyroid carcinoma, the particulate I^{131} was increased.

VI. Immunologic studies

All specimens reacted with rabbit anti-human serum albumin (RAHSA) and with rabbit anti-human thyroglobulin (RAHTH). The line of precipitation observed on Ouchterlony plates when RAHSA reacted with thyroid supernatants was continuous with that formed when RAHSA reacted with albumin. The noniodinated pre-albumin protein in 1 congenital goiter was completely precipitated by RAHSA (16).

DISCUSSION

In each category of diseased thyroid glands (colloid goiter, adenomas, congenital goiter, carcinoma) some specimens contained a noniodinated protein with the electrophoretic property of albumin. In these glands there was also a general increase in $S_{w,20}=4$ protein, most obviously in the thyroids which held the pre-thyroglobulin electrophoretic component. Further, the salting out of iodinated protein in the same glands occurred at increased phosphate buffer concentration. All gland extracts "cross-reacted" with RAHSA. These data indicate that a protein having electrophoretic, sedimentation and immunologic qualities similar to serum albumin is generally, but variably, increased in diseased thyroid tissue. Perhaps the protein is albumin. It was not possible to correlate the presence of this component with the hemoglobin content of the homogenate, which varied from near zero to 0.1 Gm. per cent, or with the vascularity of the gland. There was a correlation with the histologic structure, in that the tissues having the most obvious accumulation of this albumin-like protein (colloid goiters, 1 follicular adenoma, 1 congenital goiter), had a histologic picture of very large colloid-filled follicles. In 1 specimen this protein was present at 5 Gm. per cent concentration. Other plasma proteins were not present in proportional concentration, if at all. Further, a similar protein has been reported as a normal thyroid constituent (23). Serum albumin could have entered these glands either in extracellular fluid or from hemorrhage into a cyst. The nature and significance of this protein requires further study.

The iodinated protein of the diseased glands salted out in general at higher phosphate buffer concentration than did that of normal thyroid tissue. In glands containing an increased proportion of the $S_{w,20}=4$ protein, salting out began at 1.8–2 *M* and continued gradually up to 2.2–2.6 *M*. In these glands a major portion of iodinated protein salted out between 2 and 3 *M* phosphate buffer. In only 1 instance was this variation in solu-

bility correlated with the presence of an abnormal iodinated protein that could be detected by paper electrophoresis. In all others, iodinated thyroglobulin with apparently normal electrophoretic and sedimentation characteristics was present. No special studies were made of the protein salting out between 2 and 3 *M* phosphate. The differences between these and other reported studies (15) are quite marked, and perhaps cannot be attributed to variation in technique alone. It has been observed in this laboratory that salting out of thyroglobulin at room temperature decreases thyroglobulin solubility.[1] Further, when small amounts of labeled thyroglobulin are added to unlabeled thyroglobulin, albumin, or serum at 3 per cent concentration, the salting-out curves tend to vary, and follow that of the nonlabeled protein. This is probably because of adsorption of thyroglobulin on the major protein components. Thus, a marked increase in low-weight highly soluble protein may increase the apparent solubility of thyroglobulin. It is possible, although entirely speculative, that alteration in proportions of iodinated amino acids in thyroglobulin (increased iodotyrosine/iodothyronine ratio) may also affect its solubility. Characterization of the protein salting out between 2 and 3 *M* concentration as a protein separate and distinct from thyroglobulin could not be achieved in this study; in fact, the opposite was suggested. A small amount of a low-molecular weight, highly soluble iodinated protein has been reported by others as a normal component of the thyroid (15). The latter protein may represent iodinated protein other than the thyroglobulin normally attached to cell particles (18).

The ultracentrifuge studies on diseased glands demonstrated a general increase in the ratio of $S_{w,20}=4$ protein to $S_{w,20}=19$ protein. In some colloid goiters, some adenomas, and congenital goiters, this was due to an increased content of $S_{w,20}=4$ component, whereas in other congenital goiters and in thyroid carcinoma, the altered ratio was due to an absolute decrease in thyroglobulin content. In 1 congenital goiter, there was no thyroglobulin. There was no indication of abnormally sized or shaped thyroglobulin molecules, in that sedimentation constants of the abnormal gland proteins were the same as those of normal glands.

Within the limits of precision of technique, there was no alteration in the electrophoretic mobility of thyroglobulin in any of the glands studied. No glycoprotein other than thyroglobulin was present on electrophoretograms of the supernatant fractions.

In 1 congenital goiter and the thyroid carcinoma there was an increase in the proportion of total homogenate protein associated with the particulate fractions, and similarly in the proportion of total I^{131} present in these

[1] Data of Dr. M. J. Spiro.

particulate fractions. This correlated with a decreased content of thyroglobulin in the gland, and cannot be interpreted as of other significance. Some further definition has been made of the particulate protein. Human *in vivo* labeled mitochondria, microsomes, and nuclei-debris fraction, when incubated *in vitro* with desoxycholate, release into the media a soluble iodinated protein distinct from thyroglobulin (18). The data at hand, however, do not prove any accumulation of this or other protein in the particulate fraction in disease states.

General patterns in the foregoing data are compatible with established concepts of thyroid pathophysiology. Most parameters studied in the adenomas were similar to findings in normal glands. In contrast, the congenital goiters and carcinoma differed widely, in a fashion explicable in terms of deficient synthesis of thyroglobulin, with a resultant increase in the proportions of particulate protein and I^{131}. The accumulation of soluble noniodinated protein apparently similar to albumin in colloid goiters, some adenomas, and 1 congenital goiter, was not previously recognized. Most likely this material accumulates because of degenerative changes in the gland. Alternatively, it is possible that this excess of non-thyroglobulin protein is related to the pathogenesis of colloid goiter. Perhaps an albumin-like protein is normally deposited and removed from the colloid simultaneously with thyroglobulin. A deficient mobilization of this protein could lead to distention of the follicles and the development of the histologic picture of colloid goiter.

Acknowledgments

The authors wish to acknowledge the advice and support generously given by Dr. John B. Stanbury. The ultracentrifuge studies were kindly performed by Dr. Karl Schmid. Appreciation is also given Drs. Farahe Maloof and Oliver Cope, who helped secure thyroid specimens; to Dr. William McDermott, who performed thyroid biopsy in 2 cases; and to Miss E. Callahan for secretarial assistance.

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REFERENCES

1. Brand, E.; Kessell, B., and Heidelberger, M.: On the structure of thyroglobulin, *J. Biol. Chem.* **128**: xi, 1939.
2. Heidelberger, M., and Pedersen, K. O.: The molecular weight and isoelectric point of thyroglobulin, *J. Gen. Physiol.* **19**: 95, 1935.
3. Heidelberger, M.: The molecular weight of thyroglobulin, *Science*, **80**: 414, 1934.
4. Derrien, Y.; Michel, R., and Roche, J.: Recherches sur la preparation et les propriétés de la thyroglobuline pure. I., *Biochim. et biophys. acta* **2**: 454, 1948.
5. Derrien, Y.; Michel, R.; Pedersen, K., and Roche, J.: Recherches sur la preparation et sur les propriétés de la thyroglobuline pure. II., *Biochim. et biophys. acta* **3**: 436, 1949.

6. PITT-RIVERS, R.; HUBBLE, D., and HOATHER, W. H.: A chromatographic study of thyroidal iodine metabolism in nontoxic nodular goiter, *J. Clin. Endocrinol. & Metab.* **17:** 1313, 1957.
7. STANLEY, P. G.: Iodine-containing proteins of normal and abnormal human thyroid tissue, *Biochem. J.* **63:** 581, 1956.
8. EASTY, G. C.; SLATER, B. R., and STANLEY, P. G.: The purity of thyroglobulin isolated from normal and carcinomatous thyroid tissue on one patient by fractional salting-out with ammonium sulphate, *Biochem. J.* **68:** 210, 1958.
9. WITEBSKY, E.; ROSE, N. R., and SHULMAN, S.: Studies of normal and malignant tissue antigens, *Cancer Research* **16:** 831, 1956.
10. ROBBINS, J.; RALL, J. E., and RAWSON, R. W.: A new serum iodine component in patients with functional carcinoma of the thyroid, *J. Clin. Endocrinol. & Metab.* **15:** 1315, 1955.
11. CAMERON, C., and FLETCHER, K.: An iodine compound associated with albumin in the plasma of thyrotoxic patients, *Nature* **183:** 116, 1959.
12. LARSON, F. C.; DEISS, W. P., and ALBRIGHT, E. C.: Radiochromatographic identification of thyroxin in an alpha globulin fraction of serum separated by starch zone electrophoresis, *J. Clin. Invest.* **33:** 230, 1954.
13. DeGROOT, L. J.; POSTEL, S.; LITVAK, J., and STANBURY, J. B.: Peptide-linked iodotyrosines and iodothyronines in the blood of a patient with congenital goiter, *J. Clin. Endocrinol. & Metab.* **18:** 158, 1958.
14. DiGEORGE, A. M., and PASCHKIS, K. E.: Sporadic hypothyroidism associated with goiter, *J. Clin. Endocrinol. & Metab.* **17:** 645, 1957.
15. ROBBINS, J.; WOLFF, J., and RALL, J. E.: Iodoproteins in normal and abnormal human thyroid tissue and in normal sheep thyroid, *Endocrinology* **64:** 37, 1959.
16. DeGROOT, L. J., and STANBURY, J. B.: The syndrome of congenital goiter with butanol-insoluble serum iodine, *Am. J Med.* **27:** 586, 1959.
17. ROBBINS, J.; WOLFF, J., and RALL, J. E.: Iodoproteins in thyroid tissue and blood of rats with a transplantable thyroid tumor, *Endocrinology* **64:** 12, 1959.
18. DeGROOT, L. J.: Iodide binding in thyroid cellular fractions, *J. Biol. Chem.* (in press) March, 1960.
19. ROBBINS, J.: Thyroglobulin in serum after I^{131} therapy. I. Salting out, *J. Biol. Chem.* **208:** 377, 1954.
20. GORNALL, A. G.; BARDAWILL, C. J., and DAVID, M. M.: Determination of serum proteins by means of the biuret reaction, *J. Biol. Chem.* **177:** 571, 1949.
21. BOAS, N. F., and FOLEY, J. B.: Electrophoretic studies on the distribution of hexosamine in proteins of the thyroid gland, *Endocrinology* **56:** 474, 1955.
22. SHULMAN, S.; ROSE, N. R., and WITEBSKY, E.: Studies on organ specificity. III. Ultracentrifugal and electrophoretic examinations of thyroid extracts, *J. Immunol.* **75:** 291, 1955.
23. SHULMAN, S.; ROSE, N. R., and WITEBSKY, E.: Thyroalbumin—a new antigen from thyroid tissue, *Fed. Proc.* **16:** 433, 1957.

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OXYGEN CONSUMPTION AND THYROXINE DEIODINATION BY HUMAN LEUKOCYTES*†

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ABSTRACT

Homogenates of white blood cells from 31 euthyroid persons, 16 thyrotoxic patients and 3 hypothyroid patients were incubated with labeled thyroxine. Respiration and deiodination of thyroxine were measured. The oxygen consumption of white-cell homogenates from 31 euthyroid subjects ranged from 0.150 to 0.545 and averaged 0.334 ± 0.1 μl. $O_2/10^6$ cells/hour. Oxygen consumption by leukocytes from thyrotoxic patients was significantly increased, and in 16 thyrotoxic subjects ranged from 0.21 to 0.76 and averaged $0.444 \pm .15$ μl. $O_2/10^6$ cells/hour. The oxygen consumption by leukocytes from 3 hypothyroid subjects averaged 0.313 μl. $O_2/10^6$ cells/hour. Deiodination of labeled thyroxine by cells from 10 euthyroid women ranged from 7 to 39 per cent of the total radioactivity and averaged 19 ± 16 per cent. Deiodination by cells from 7 thyrotoxic women was significantly greater, and ranged from 15 to 57 per cent, averaging 44 ± 14.5 per cent. The average deiodination by leukocytes from 3 hypothyroid patients was only 7.3 per cent—below the euthyroid mean. The deiodinase of human leukocytes is active at physiologic pH 7.4, is thermolabile (being decreased by heating to 60–70° C. for thirty minutes), and is aerobic (being inactive in an atmosphere of nitrogen). It is inhibited by 10^{-3} NaCn and 0.1 *M* $HgCl_2$, and requires both ATP and DPN for maximum activity. The use of the leukocyte as a test organ may permit direct studies of peripheral thyroid hormone metabolism in man.

IN CONTRAST to extensive data on thyroid gland function, data on peripheral thyroid hormone metabolism in man are few and largely indirect, limited by the inaccessibility of many tissues.

The present communication reports an attempt to make direct studies of peripheral thyroid metabolism by measurement of oxygen consumption and thyroxine metabolism in leukocytes from euthyroid and hyperthyroid persons.

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MATERIALS AND METHODS

White blood cells were obtained from 31 euthyroid subjects (17 women and 14 men), 16 thyrotoxic patients (13 women and 3 men) and 3 hypothyroid patients. The metabolic status of all subjects was ascertained by clinical evaluation and confirmed by determinations of the basal metabolism, serum protein-bound iodine level, 24-hour thyroidal uptake of radioiodine (I^{131}), or the two-hour erythrocyte uptake of triiodothyronine (1).

Leukocytes were isolated after sedimentation with dextran, according to the following method:[1] 1.5 ml. of 20 per cent dextran (Commercial Solvents, Lot No. NA 649) and 0.9 ml. of 5 per cent Sequestrene[2] were added under sterile conditions to a 50-ml. syringe. Venepuncture was made with minimal trauma under venous stasis and a 50–200 ml. sample of blood was collected. The syringe was inverted twenty times to insure mixing and was clamped upright in the cold (6–8° C.). After settling for one and a half to two hours, the supernatant plasma containing the leukocytes was expressed into a cold 50-ml. Lusteroid centrifuge tube and spun for five minutes at 1000 rpm at 4° C. Erythrocytes and some white cells came down in this spinning. The supernatant plasma was transferred to a cold Lusteroid tube and spun for ten minutes at 1200 rpm at 4° C. The resulting supernatant was centrifuged a third time at 1500 rpm for ten minutes at 4° C. One ml. of Hank's solution (2) and 10.0 units of Varidase[3] were added to each tube after the plasma had been removed, and the white cells were resuspended with a pipette. The white cells were combined, centrifuged and washed three times with 35–40 ml. of Hank's solution. Total and differential white cell counts were performed. From 57 to 150 million white cells were obtained from 50 ml. of blood. From 67 to 93 per cent (average, 85) were polymorphonuclear leukocytes. The final ratio of red to white cells ranged from 4:1 to 1:1, and the ratio of platelets to white cells was approximately 4:1. The cells were homogenized by hand in the cold for four minutes with a Teflon homogenizer. Approximately 40 to 70 per cent of the cells were broken. In duplicate samples, the percentage of broken cells varied with narrow limits.

The homogenate of white cells was added to an incubation mixture (3) (Table 1). I^{131}-labeled thyroxine (7 to 9 microcuries) was added to yield a final concentration of 10^{-8} to 10^{-9} *M* thyroxine. Respiration was measured by the direct method of Warburg, often in duplicate or quadruplicate (4). At the end of sixty minutes, aliquots were removed, suitable stable carriers were added, and 0.02 ml. was spotted directly onto filter paper and analyzed by ascending chromatography in three systems: (a) tertiary amyl alcohol:ammonium hydroxide, (b) butanol:ammonium hydroxide, and (c) butanol: acetic acid:water (78:10:12). The dried chromatograms were stained with diazotized sulphanilic acid and 0.5 per cent palladium chloride to locate carriers. Radioactivity was located by a Geiger-Müller tube with an automatic scanning device. The area under each curve of radioactivity was measured by planimetry and expressed as a percentage of the total radioactivity on the chromatogram.

In each case, a control mixture of labeled thyroxine and all other ingredients, except cells, was incubated simultaneously. Thyroxine obtained from a commercial source[4] contained 2 to 15 per cent iodide prior to the study. Additional deiodination of thyroxine was noted in the control flask. The net iodide resulting from the action of white cells

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[1] Suggested by Dr. W. C. Moloney.

[2] Sequestrene Sodium (Geigy).

[3] Added at the suggestion of Dr. J. L. Tullis. Varidase (Lederle) is a combination of streptokinase and streptodornase, used for enzymolytic removal of necrotic tissue.

[4] Abbott Laboratories, North Chicago, Ill.

was obtained by subtracting the percentage of iodide on the chromatogram of the control material from that produced by the cells. The percentage thyroxine deiodination by white blood cells in a given experiment was remarkably similar in all chromatographic systems used. When red blood cells were incubated with thyroxine^{131} and an aliquot spotted directly and chromatographed, no radioactivity moved from the origin.

RESULTS

The oxygen consumption of homogenates of white blood cells from 31 euthyroid subjects ranged from 0.150 to 0.545 and averaged $.334 \pm 0.2$ μl. of oxygen per 10^6 cells per hour ($O_2/10^6$ cells/hour). The cells of 17 euthyroid women averaged 0.327 μl. $O_2/10^6$ cells/hour and those of 14 men

TABLE 1. INCUBATION MIXTURE FOR HOMOGENATE OF WHITE BLOOD CELLS

Material	μM added
$MgCl_2$	15.0
ATP*	3.0
DPN†	2.0
PO_4 buffer, pH 7.4	104.0
Glucose	15.0
Cytochrome C	0.036

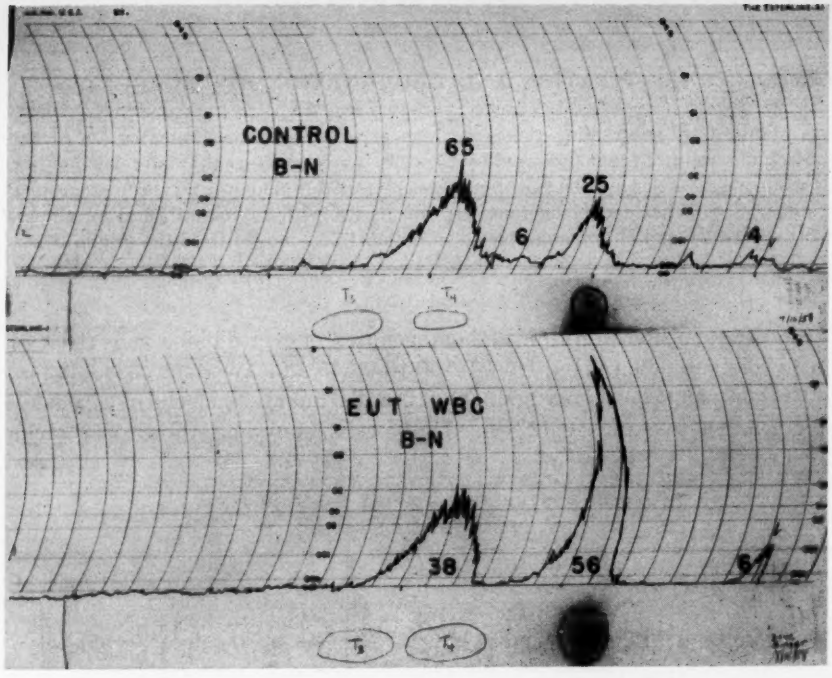
All solutions except $MgCl_2$+WBC were made up in PO_4 buffer. Incubation mixture was added to 1.6 ml. of WBC to yield a final volume of 3.3 ml.

* Adenosine triphosphate.

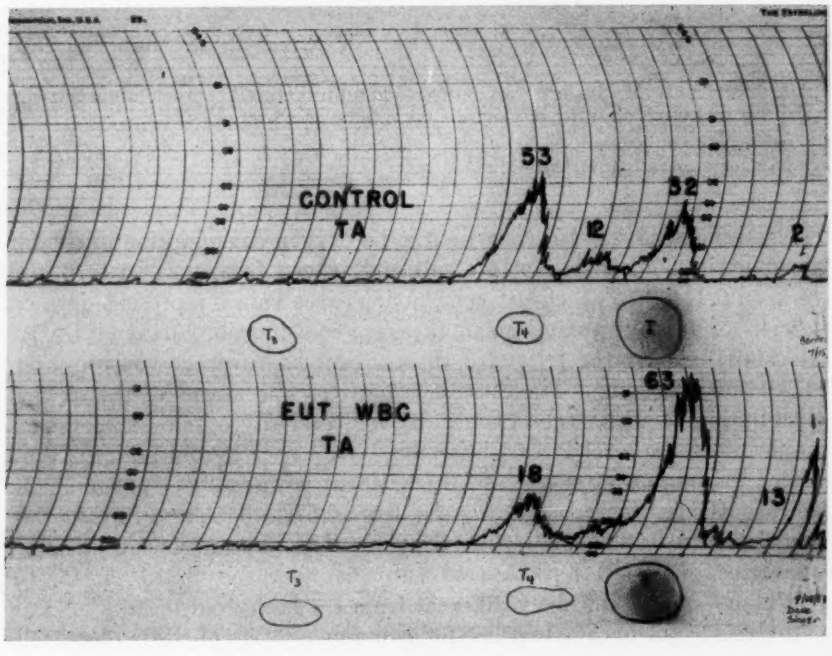
† Diphosphopyridine nucleotide.

averaged 0.346 μl. $O_2/10^6$ cells/hour. The oxygen consumption in similar determinations on 16 thyrotoxic subjects ranged from 0.21 to 0.76 and averaged $0.444 \pm .15$ μl. $O_2/10^6$ cells/hour. These values represent data on the cells of 13 thyrotoxic women (average consumption, 0.451 μl. $O_2/10^6$ cells/hour), and on the cells of 3 thyrotoxic men (average consumption, 0.478 μl. $O_2/10^6$/hour). The difference between the means of the oxygen consumption of white cells from euthyroid and thyrotoxic subjects was significant at the level $P > .01$ but $< .02$. Two hyperthyroid patients were studied before and after successful treatment with radioactive iodine. In one case, the oxygen consumption fell from 0.57 to 0.37, and in the other from 0.400 to 0.136 μl. $O_2/10^6$ cells/hour. The oxygen consumption by leukocytes of the 3 hypothyroid subjects averaged 0.313 μl. $O_2/10^6$ cells/hour—not significantly different from the euthyroid mean.

Analysis of the chromatograms following incubation of white blood cells with labeled thyroxine demonstrated that white cells are capable of deiodi-



a



b

FIG. 1. The deiodination of thyroxine by leukocytes. Upper chromatogram—control. Lower chromatogram—cells from euthyroid subject. The percentage of total radioactivity is noted above each peak. Abbreviations: T_4 = thyroxine; T_3 = triiodothyronine; I^- = iodide. **a**) butanol:ammonium hydroxide system. **b**) tertiary amyl alcohol:ammonium hydroxide system.

nating thyroxine (Fig. 1 a and b). In most cases, only thyroxine and iodide were identified. Rarely, a small amount of radioactivity corresponding to triiodothyronine in chromatographic mobility was noted.

Deiodination by the leukocytes from 10 euthyroid women ranged from 7 to 39 per cent of the total radioactivity and averaged 19 ± 16 per cent. Deiodination by the leukocytes from 7 thyrotoxic women ranged from 15 to 57 per cent of the total radioactivity and averaged 44 ± 14.5 per cent (Fig. 2). The difference was statistically significant, $P > .001$ but $< .005$. Deiodination by white cells from 7 euthyroid men averaged 28 ± 10.5 per cent of the radioactivity. Studies were performed on only 2 thyrotoxic men, and the average deiodination by white cells was 24 per cent. One thyrotoxic subject was studied again when euthyroid; deiodination by leukocytes had fallen from 32 to 18 per cent. The average deiodination by leukocytes of the 3 hypothyroid patients was only 7.3 per cent below the euthyroid value.

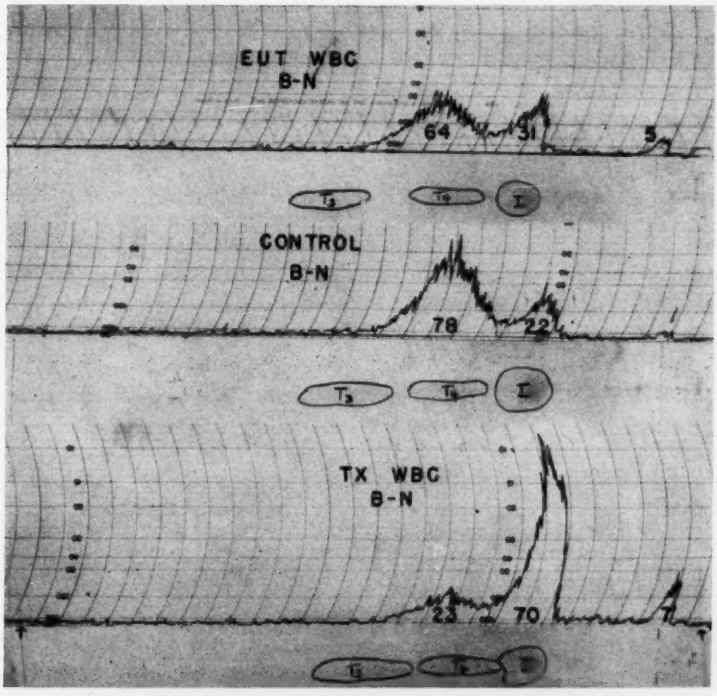


FIG. 2. Deiodination of thyroxine by leukocytes from a euthyroid and from a thyrotoxic subject. Upper chromatogram—leukocytes from euthyroid subject. Middle chromatogram—control. Lower chromatogram—leukocytes from thyrotoxic subject. System—butanol:ammonium hydroxide.

TABLE 2. EFFECT OF HOMOGENIZATION ON DEIODINATION BY LEUKOCYTES (WBC)

Subject	Sex	State of WBC	% I formed greater than in control
KL	M	Homog.	22
		Nonhomog.	0
AG	M	Homog.	30
		Nonhomog.	8

Summary of the properties of the deiodinating system of human white blood cells

1. Minimal deiodination of I^{131}-labeled thyroxine resulted from the use of intact white cells in contrast to the homogenized cells from the same subject (Table 2).

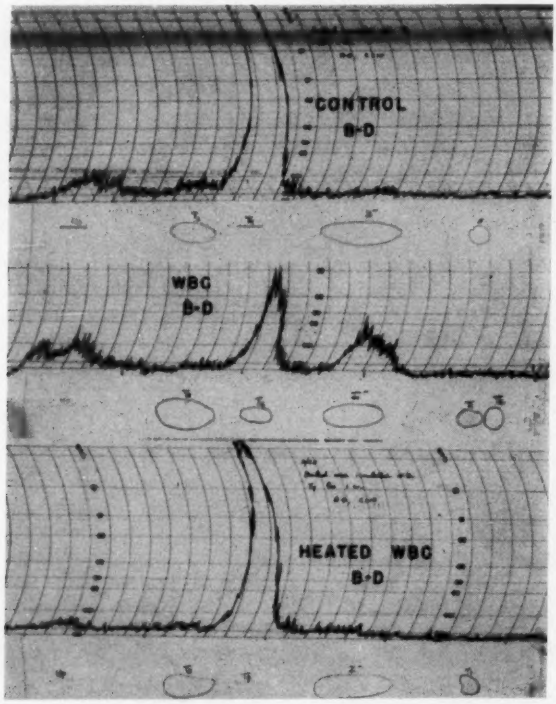


FIG. 3. Deiodination of thyroxine by normal and by boiled leukocytes. Upper chromatogram—control without cells. Middle chromatogram—normal white blood cells. Lower chromatogram—heated cells. System—butanol:dioxane.

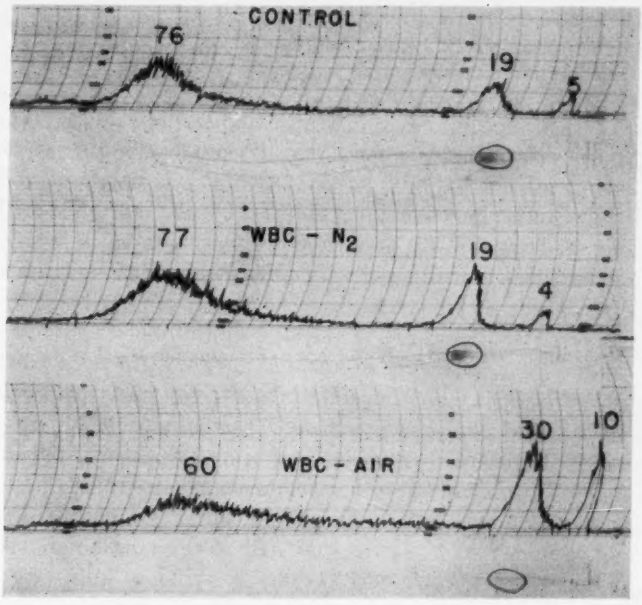


FIG. 4. Effect of nitrogen on deiodination of thyroxine by leukocytes. Upper chromatogram—control. Middle chromatogram—incubation under nitrogen. Lower chromatogram—incubation under air. System—butanol:acetic acid.

2. Heating the cells to 60–70° C. for thirty minutes diminished deiodination. Boiling the cells for thirty minutes eliminated deiodination (Fig. 3).

3. Anaerobic incubation under nitrogen eliminated deiodination (Fig. 4).

4. Both adenosine triphosphate (ATP) and diphosphopyridine nucleotide (DNP) were required for maximum respiration and deiodination. Respiration and deiodination were markedly depressed by eliminating ATP and DPN from the incubation mixture (Tables 3 and 4); omission of DPN alone resulted in a substantial decrease of respiration and deiodination; absence of ATP resulted in a marked decrease in deiodination but only a slight decrease in respiration.

5. The addition of 10^{-2} *M* sodium fluoride (NaF) stimulated respiration but had no effect on deiodination (Tables 3 and 4); 10^{-3} *M* sodium cyanide (NaCn) prevented deiodination but did not stop respiration; .011 *M* mercuric chloride ($HgCl_2$) eliminated both respiration and deiodination.

6. In time-course studies, deiodination was present in fifteen minutes and increased rapidly, reaching a peak between two and four hours; it had decreased by eighteen hours.

7. Evidence for deiodination of triiodothyronine by leukocytes from hyperthyroid or euthyroid subjects was obtained in only 1 of 4 experi-

TABLE 3. EFFECT OF OMISSION OF ATP AND DPN SIMULTANEOUSLY, AND ADDITION OF NaCN, ON O_2 CONSUMPTION AND DEIODINATION OF THYROXINE BY WHITE BLOOD CELL HOMOGENATES

Change in WBC homogenate	Oxygen consumption (μl. $O_2/10^6$ WBC)	% I formed		
		B–N*	B–HAC	Avge.
	—	46	48	47
+NaF	0.425	47	31	39
−ATP, −DPN	.017	4	7	5
+NaCN	.295	2	6	4

* Solvent systems (see text): B–N = butanol:ammonia; B–HAC = butanol:acetic acid.

TABLE 4. EFFECT OF OMISSION OF DPN AND ATP AND ADDITION OF NaF ON O_2 CONSUMPTION AND DEIODINATION OF THYROXINE BY WHITE BLOOD CELL HOMOGENATES

Change in WBC homogenate	Oxygen consumption (μl. $O_2/10^6$ WBC)	% I formed			
		TA*	BN	B–HAC	Avge.
	.475	36	42	42	40
−DPN	.110	23	19	19	20
−ATP	.316	14	19	5	13
+NaF	.610	46	52	41	46

* Solvent systems (see text): TA = tertiary amyl alcohol-ammonia; B–N = butanol-ammonia; B–HAC = butanol:acetic acid.

ments. No evidence for deiodination of the acetic analogues of thyroxine or triiodothyronine was found.

DISCUSSION

Our studies demonstrate an increased oxygen consumption by leukocytes from thyrotoxic persons compared to those of euthyroid persons. The data show further that human white blood cells contain an enzyme(s) capable of deiodinating thyroxine and that the white blood cells from thyrotoxic women induce greater deiodination of thyroxine than cells derived from euthyroid women.

The significance of these observations and the usefulness of this technique in studying metabolic states rest in part on the extent to which the metabolism of leukocytes is representative of that of other tissues of the body. Other fresh human tissues have not been available to us, and thus

comparison of the metabolism of thyroxine by white blood cells and by other tissues has not been possible.[5]

It has previously been shown that the circulating leukocyte contains many enzymes and participates in metabolic abnormalities in several human diseases (6). The increased respiration of leukocytes from thyrotoxic subjects encouraged the view that these cells participate in the metabolic changes in abnormal thyroid states. Evidence suggesting that human tissues are capable of deiodinating thyroxine, as indicated by the discovery of labeled triiodothyronine following the infusion of thyroxine, has not been confirmed (7, 8). However, indirect evidence confirming thyroxine deiodination has been obtained (9–11) in studies showing iodide excretion in the urine after intravenous infusion of I^{131}-labeled thyroxine. Although tissue studies in man are not available, previous reports by others (summarized in Table 5) indicate that animal tissues metabolize thyroxine in a variety of ways. The variety of methods (deiodination, deamination) by which different tissues metabolize thyroxine has also been demonstrated following injection of I^{131} into guinea pigs (21). In comparison to the foregoing, the deiodinase of human leukocytes, like the deiodinase of rat kidney and brain, is active at physiologic pH 7.4, is thermolabile, is destroyed by heating to 60–70° C. for thirty minutes, is aerobic (being inactivated by an atmosphere of nitrogen), and is inactivated by .011 *M* mercuric chloride. On the other hand, unlike brain deiodinase (18), it is entirely inhibited by 10^{-3} sodium cyanide but not at all by sodium fluoride. Like the enzyme of muscle homogenates, the major reaction product is iodide. Unlike the rat kidney enzyme system which converts thyroxine to its acetic-acid analogues, both ATP and DPN were necessary cofactors for full deiodination of thyroxine by our white cell homogenates. It should be be noted that consistent deiodination of triiodothyronine by human leukocytes was not demonstrated. Thus, human leukocyte deiodinase resembles the thyroid hormone metabolism system found in some animal tissues but not in others. In view of the results of animal studies, it is probably representative of one of several possible metabolic pathways.

We have previously postulated abnormalities of the peripheral metabolism of thyroxine in describing the role of altered hormone function in euthyroid hypometabolism and in paroxysmal atrial arrhythmias (11, 22). This hypothesis was supported by indirect studies of peripheral thyroxine metabolism as measured by the rate of disappearance of intravenously infused I^{131}-labeled thyroxine. It is hoped that the present technique will make feasible direct studies of peripheral thyroid metabolism in these and other states of altered thyroid metabolism. This possibility has been

[5] In a recent abstract (5), the conversion of triiodothyronine to triiodothyroacetic acid (triac) by human liver and kidney has been described.

TABLE 5. METABOLISM OF THYROXINE BY ANIMAL TISSUES

Author	Reference No.	Animal	Organ	Substrate*	Product	pH	Aerobic or anaerobic	Inhibitors	Heat stability	Comment
Albright *et. al.* Larson *et al.*	(12) (13)	Rat	Kidney slice	Thy^{131}	Tri^{131}	7.4		KCN I Thiouracil	Inactivated by boiling 5 mins.	
Tomita *et al.* Albright *et al.*	(14) (15)	Rat	Mitochondria from kidney homogenate; also liver, heart, spleen, brain	Thy^{131} Tri^{131} Tri^{131}	$Tetrac^{131}$ $Triac^{131}$ Iodide	7.4			Inactivated by boiling 10 mins.	Sonic disruption of mitochondria increased. Triac formation from Tri.
Tata	(16)	Rat	Skel. muscle	Thy^{131}	Iodide		Either		Inactivated by 100°C. for 20 mins.	10–15% acetic or propionic analogues.
Tata *et al.*	(18)	Rat Chick Dog	Brain slice or conc. homog. Dilute homog.	Thy^{131} Tri^{131} Tri^{131}	Iodide Iodide Propionic or acetic analogues	7.4	Either	NaCN 2-octanol $HgCl_2$	Inactivated by 100°C. for 20–30 mins.	
Yamazaki and Slingerland	(17)	Rat	Kidney, liver, spleen, muscle slices; homog. or fractions of homog.	Thy^{131} Tri^{131} Tetrac Triac	Iodide Iodide Iodide Iodide	7.4	Aerob.	Metals Cyanide 2-Methyl-1,4-naphthoquinone Versene Estrogen BHDB,† $HgCl_2$	Slices inactivated by boiling. Homog., 30 mins. boiling, no effect	
Maclagan and Sprott	(19) (20)	Rat	Homog., esp. liver; also brain, kidney, muscle, spleen, adrenal, thyroid	Stable Thy Stable Tri Thy^{131}	Iodide ? Tri Iodide I^{131}	3.5 and 9.5 9.5 5 to 7	Aerob. Aerob.	KCN KCN BHDB†	Resistant to 100°C. for 30 mins. Inactivated by 100°C. for 2 hrs.	

* Thy = thyroxine; Tri = triiodothyronine; Triac = triiodothyroacetic acid; Tetrac = tetraiodothyroacetic acid.
† BHDB = Butyl 4-hydroxy-3:5-diiodobenzoate.

strengthened by the finding that the oxygen consumption of leukocytes is increased in thyrotoxicosis and that this increase in oxygen consumption parallels the increase in general body metabolism (23). Increased deiodination of thyroxine by leukocytes from thyrotoxic subjects is further evidence of the participation of white cells in the metabolic changes in thyrotoxicosis. Our data are in agreement with those obtained in experiments showing increased deiodinating activity in hyperthyroid rats (13); other animal experiments have yielded negative results (24). The ultimate usefulness of the leukocyte as a test organ for direct study of thyroid hormone metabolism in man thus awaits further investigation.

REFERENCES

1. Hamolsky, M. W.; Golodetz, A., and Freedberg, A. S.: The plasma protein-thyroid hormone complex in man. III. Further studies on the use of the *in vitro* red blood cell uptake of I^{131}-*l*-triiodothyronine as a diagnostic test of thyroid function, *J. Clin. Endocrinol. & Metab.* **19:** 103, 1959.
2. McKinney, G. R.; Martin, S. R., and Rundles, R. W.: Respiratory and glycolytic activities of human leukocytes in vitro, *J. Appl. Physiol.* **5:** 335, 1953.
3. Beck, W. C., and Valentine, W. N.: The aerobic metabolism of leukocytes in health and leukemia. I. Glycolysis and respiration, *Cancer Research* **12:** 818, 1952.
4. Umbreit, W. W.; Burris, R. H., and Slauffer, J. F.: Manometric Techniques and Tissue Metabolism. Minneapolis, Burgess Publishing Co., 1949.
5. Albright, E. C.; Tomita, K., and Larson, F. C. Metabolism of L-triiodothyronine by human mitochondria, (abstract) *J. Clin. Invest.* **38:** 984, 1959.
6. Martin, S. P.: Methods for the study of human leukocytes, *in* Methods in Medical Research, ed. by J. V. Warren. Chicago Ill., Yearbook Publishers, 1958, Vol. 7, Section 3.
7. Pitt-Rivers, R.; Stanbury, J. B., and Rapp, B.: Conversion of thyroxine to 3-5-3′-triiodothyronine *in vivo*, *J. Clin. Endocrinol. & Metab.* **15:** 616, 1955.
8. Lassiter, W. E., and Stanbury, J. B.: The *in vivo* conversion of thyroxine to 3:5:3′-triiodothyronine, *J. Clin. Endocrinol. & Metab.* **18:** 903, 1958.
9. Ingbar, S. H., and Freinkel, H.: Simultaneous estimation of rates of thyroxine degradation and thyroid hormone synthesis, *J. Clin. Invest.* **34:** 808, 1955.
10. Sterling, K., and Chodos, R. B.: Radiothyroxine turnover studies in myxedema, thyrotoxicosis, and hypermetabolism without endocrine disease, *J. Clin. Invest.* **35:** 806, 1956.
11. Kurland, G. S.; Bustos, J. G.; Hamolsky, M. W., and Freedberg, A. S.: Studies in nonmyxedematous hypometabolism. II. Turnover of I^{131}-labeled thyroxine after intravenous infusion, *J. Clin. Endocrinol. & Metab.* **17:** 1365, 1957.
12. Albright, E. C.; Larson, F. C., and Tust, R. H.: In vitro conversion of thyroxin to triiodothyronine by kidney slices, *Proc. Soc. Exper. Biol. & Med.* **86:** 137, 1954.
13. Larson, F. C.; Tomita, K., and Albright, E. C.: The deiodination of thyroxine to triiodothyronine by kidney slices of rats with varying thyroid function, *Endocrinology* **57:** 338, 1955.
14. Tomita, K.; Lardy, H. A.; Larson, F. C., and Albright, E. C.: Enzymatic conversion of thyroxine to tetraiodothyroacetic acid and of triiodothyronine to triiodothyroacetic acid, *J. Biol. Chem.* **224:** 387, 1957.

15. ALBRIGHT, E. C.; TOMITA, K., and LARSON, F. C.: *In vitro* metabolism of triiodothyronine, *Endocrinology* **64**: 208, 1959.
16. TATA, J. R.: Metabolism of *l*-thyroxine and *l*-3:5:3′-triiodothyronine by homogenates of rat skeletal muscle, *Proc. Soc. Exper. Biol. & Med.* **93**: 362, 1957.
17. YAMAZAKI, E., and SLINGERLAND, D. W.: The *in vitro* metabolism of thyroxine, triiodothyronine and their acetic and propionic acid analogues, *Endocrinology* **64**: 126, 1959.
18. TATA, J. R.; RALL, J. E., and RAWSON, R. W.: Metabolism of L-thyroxine and L-3:5:3′ triiodothyronine by brain tissue preparations, *Endocrinology* **60**: 83, 1957.
19. MACLAGAN, N. F., and SPROTT, W. E.: The in-vitro deiodination of thyroxine and triiodothyronine, *Lancet* **2**: 368, 1954.
20. SPROTT, W. E., and MACLAGAN, N. F.: Metabolism of thyroid hormones: the deiodination of thyroxine and triiodothyronine in vitro, *Biochem. J.* **59**: 288, 1955.
21. FORD, D. H.; COREY, K. R., and GROSS, J.: The localization of thyroid hormones in the organs and tissues of the guinea pig: an autoradiographic and chromatographic study, *Endocrinology* **61**: 426, 1957.
22. KURLAND, G. S.; GOLODETZ, A.; HAMOLSKY, M. W., and FREEDBERG, A. S.: Thyroid function in supraventricular tachycardias, *J. Clin. Endocrinol. & Metab.* **19**: 92, 1959.
23. ALEXANDER, W. D., and BISSET, S. K.: The influence of thyroid function on the respiration of leukocytes in vitro. Abstr. 9–22, Proc. 4th Internat. Congress of Biochemistry, 1958, p. 105.
24. MACLAGAN, N. F., and REID, D.: The deiodination of thyroid hormones *in vitro*, *in* Regulation and Mode of Action of Thyroid Hormones, ed. by G. Wolstenholme and E. Millar. Volume X, Ciba Foundation Colloquia on Endocrinology. Boston; Little, Brown and Co., 1957, pp. 190–199.

RESPONSE OF THE THYROID GLAND TO THIOCYANATE AND THYROTROPIN*†

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ABSTRACT

In order to determine whether inhibition of the thyroidal iodide "trap" by thiocyanate was dependent only upon the serum concentration of the ion, thyroid function was studied in 15 euthyroid and 3 hyperthyroid subjects before and during administration of potassium thiocyanate. Serum thiocyanate levels were correlated with thyroidal radioiodine (I^{131}) uptakes, and thyrotropin was administered to 8 euthyroid and 3 hyperthyroid subjects while the thyroid gland was under the inhibitory influence of thiocyanate. In euthyroid subjects complete interference with thyroidal I^{131} accumulation did not occur readily at serum thiocyanate levels below 5 mg. per 100 ml. In hyperthyroid subjects, thyroidal I^{131} uptake proved impossible to inhibit completely, regardless of the magnitude of the serum thiocyanate level. In euthyroid subjects, at all serum thiocyanate concentrations, thyrotropin (20 to 30 U.S.P. units) produced measurable increments in the collection of radioiodine by the thyroid. In contrast, the hyperthyroid subjects exhibited much less response to thyrotropin. Aside from the distinct differences between euthyroid and hyperthyroid subjects in their responses to thiocyanate and thyrotropin, the data suggest that the mechanism by which thiocyanate inhibits the thyroidal iodide "trap" may not be solely a function of extracellular concentration, since the thyroidal incorporation of radioiodine in response to thyrotropin may occur in the presence of an otherwise inhibitory serum level of thiocyanate.

THE ability of the thiocyanate ion to alter normal thyroid function was recognized more than two decades ago when, in several patients receiving thiocyanate therapy for hypertension, enlargement of the thyroid and features of myxedema developed (1). The goiter and myxedema in these patients could be reversed by the administration of desiccated thyroid (2). Studies in rats revealed that thiocyanate-induced goiters could be abolished by an increased iodide content of the diet (3). The suggestion was made that the favorable influence of the iodide resulted from synthesis of hormone from iodide that had diffused into the thyroid gland as a consequence of an increased serum concentration of the ion (4). Later studies

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showed that thiocyanate interfered with thyroid function by preventing the concentration of iodide by the thyroid gland (5).

The mechanism of the action of the thiocyanate ion upon the thyroidal iodide "trap" has never been satisfactorily explained. This in part may be attributed to a lack of knowledge concerning the fundamental nature of the "trap" itself. The present studies were performed in order to gain some insight into the behavior of the thyroid gland while under the influence of thiocyanate. To ascertain whether inhibition of the thyroidal iodide-collecting mechanism varies solely with the level of thiocyanate in the serum, thyroid function was examined with and without administration of thyrotropin at different serum thiocyanate concentrations.

MATERIALS AND METHODS

Ten men and 8 women, between the ages of 20 and 68, participated in this study. Fifteen were hospitalized convalescent patients who by the usual clinical and laboratory criteria were considered euthyroid, and the 3 others had unequivocal hyperthyroidism. Oral tablets of enteric-coated potassium thiocyanate[1] were administered for periods which varied from three to ten weeks. Serum thiocyanate determinations were performed by a standardized spectrophotometric method (6). Each subject received an initial loading dose of 0.8 to 1.6 Gm. of potassium thiocyanate, which was followed by 0.2 to 0.8 Gm. each day, depending upon the serum thiocyanate values. An attempt was made to maintain the level of serum thiocyanate concentration between 6.0 and 13.0 mg. per 100 ml., especially during the periods of thyrotropin administration. Single or multiple intramuscular injections of thyrotropin[2] for a total of 20 to 30 U.S.P. units were received by 8 of the euthyroid subjects and the 3 hyperthyroid patients. Thyrotropin was given, in each case, while the thyroid gland was under the inhibitory influence of the thiocyanate ion. Radioiodine was administered either twenty-four hours after the single dose of thyrotropin or on the day of the final injection of the multiple dose of thyrotropin.

The concentration of radioiodine in the thyroid gland was measured with a collimated scintillation probe at a distance of 51 cm. from the surface of the skin. In order to be able to discern slight changes in thyroid function following injection of thyrotropin, daily measurements of the radioactivity within the thyroid gland (without correction for physical decay) were plotted on semi-logarithmic paper to reflect the thyroidal secretion rate. Baseline and subsequent radioiodine uptake determinations were measured twenty-four hours after the administration of oral tracer doses of 35 to 75 microcuries of carrier-free radioiodine (I^{131}).[3] When radioiodine uptakes were determined repeatedly in the same subject, the residual thyroidal radioactivity (radioactivity remaining from a previous tracer dose) was estimated by extrapolation of the slope of thyroidal radioactivity to the time when the radioiodine uptake was determined. The residual thyroidal radioactivity, so determined, added to the body background radioactivity (which was obtained by observing the counting rate when the region of the thyroid gland was shielded with lead of $\frac{3}{4}$-inch thickness) was subtracted in the calculations made for the repeat I^{131} uptake determinations.

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[1] Enseals, 0.2-Gm. tablets, Eli Lilly and Co.

[2] Thytropar, Armour Pharmaceutical Co.

[3] Abbott Laboratories and E. R. Squibb and Sons, Oak Ridge, Tenn.

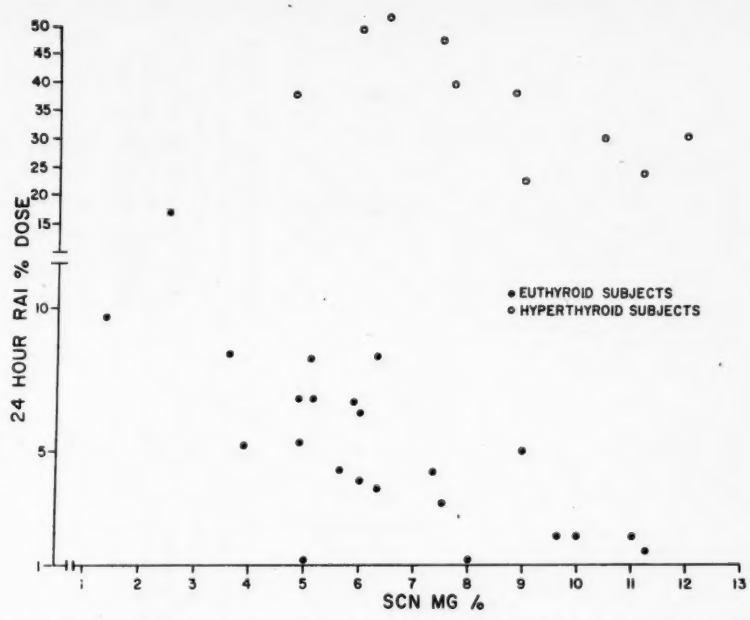

FIG. 1. Comparison in euthyroid (dots) and hyperthyroid (circles) subjects of the 24-hour thyroidal uptake of I^{131} (vertical axis) at similar serum thiocyanate concentrations (horizontal axis).

RESULTS

The correlation between serum thiocyanate concentration and 24-hour thyroidal I^{131} uptake in the euthyroid subjects is illustrated in Figure 1. A reciprocal relationship between the level of thiocyanate and the height of the radioiodine uptake appeared to obtain, especially at extremely high and low concentrations of thiocyanate in the serum. It seems reasonable to infer from examination of the plotted values that complete inhibition of thyroid function did not occur readily at serum thiocyanate levels below 5 mg. per 100 ml. Twenty-four hours after a single dose of 1.6 Gm. of potassium thiocyanate, the serum level ranged between 4.0 and 6.0 mg. per 100 ml., and diminished slowly in the ensuing days to weeks.[4]

When thyrotropin was administered to the euthyroid subjects whose thyroid glands had been effectively prevented from accumulating radioiodide by potassium thiocyanate, a variable but measurable quantity of I^{131} was collected by the thyroid glands. The details of individual studies in euthyroid Subjects T.M. and H.F. (Fig. 2) reflect the thyrotropin-in-

[4] In sera collected at random from hospitalized patients, thiocyanate concentrations have ranged from 0 to 1.5 mg. per 100 ml.

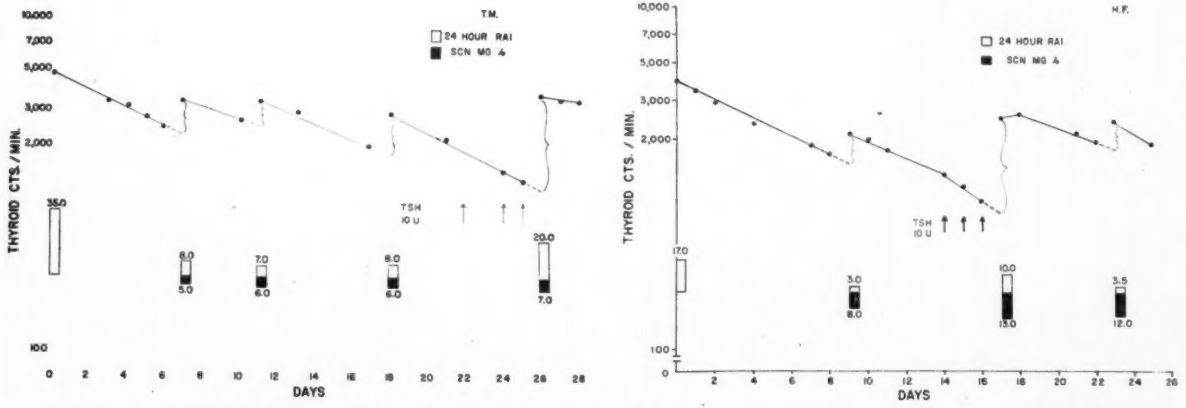

FIG. 2. The influence of thiocyanate (SCN) upon thyroidal radioiodine uptake, and the change in thyroid function after administration of thyrotropin (TSH) in euthyroid Subjects T.M. and H.F. The baseline and repeat radioiodine uptake determinations are represented by the open columns, and the values are recorded above each. The serum thiocyanate concentrations are represented by the solid black columns, and the values are printed directly beneath. Thyroidal radioactivity values are plotted on a semi-logarithmic scale (vertical axis) and joined by solid lines. The broken lines reflect extrapolated values; the bows indicate the start of the new plot following the repeat tracer dose of I^{131}. Each arrow indicates a single dose of thyrotropin.

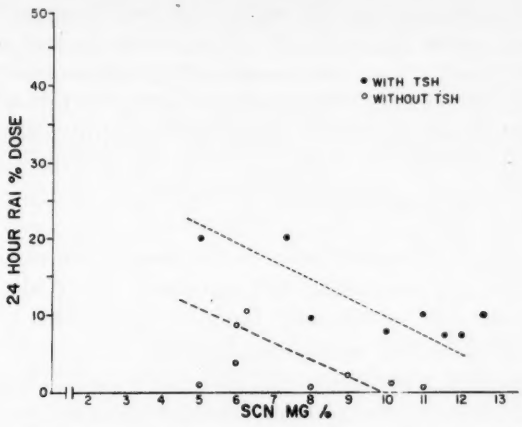

FIG. 3. Euthyroid subjects. Comparison of 24-hour thyroidal I^{131} uptakes (vertical axis) before (circles) and after (dots) the administration of thyrotropin at varying levels of serum thiocyanate concentration (horizontal axis). The mean values for each group of determinations are depicted by the sight lines.

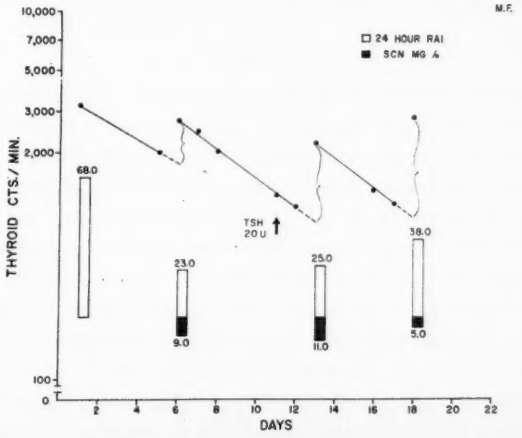

FIG. 4. Hyperthyroid Subject M.F. The influence of thiocyanate upon thyroidal radioiodine uptake and the minimal change in thyroid function after administration of thyrotropin. The baseline thyroidal I^{131} uptake is represented by the open column at the far left, with the value recorded above; additional I^{131} uptake values are shown over the corresponding open columns. Serum thiocyanate concentrations are depicted by the closed columns with the values printed beneath. Thyroidal radioactivity counts are plotted on a semi-logarithmic scale (vertical axis) and joined by solid lines. The broken lines reflect extrapolated values, and the bows indicate the start of the new plot following a repeat tracer dose of I^{131}. The arrow indicates a single dose of thyrotropin.

duced changes in thyroid function which occurred at serum levels of thiocyanate that otherwise had virtually abolished the thyroid iodide "trap." Not only was the thyrotropin effect manifested by the augmented thyroidal content of I^{131}, but the slope of thyroidal radioactivity following the repeat tracer of I^{131} remained the same or diminished on a quantitatively different plot, indicating that the I^{131} abstracted from the plasma was also bound. The 24-hour thyroidal uptakes of I^{131} at varying serum thiocyanate levels, before and after administration of thyrotropin, have been compared in Figure 3. The mean difference between the groups, expressed by the sight lines, is apparent and was statistically significant ($P < 0.01$).

In marked contrast to normally functioning thyroids in which thiocyanate had an inhibitory effect, the hyperthyroid glands proved to be resistant to complete suppression with this medication (Fig.1). Regardless of the concentration of thiocyanate, the thyrotoxic glands retained their capacity to entrap and apparently organify a considerable quantity of radioiodide. There was little or no response of the hyperthyroid glands to thyrotropin when the thyroidal radioiodine uptake values had been reduced to the normal range by appreciable concentrations of thiocyanate (Fig. 4). Table 1 summarizes the studies carried out on both the euthyroid and the hyperthyroid subjects.

DISCUSSION

From the results described, it appears that the degree of thyroid inhibition produced by the thiocyanate ion varies with its concentration in the serum. This conclusion applies equally to the euthyroid and the hyperthyroid subjects, although the latter proved to be much more refractory to the effects of thiocyanate at any given serum concentration. Although the number of observations made upon the thyrotoxic subjects was small, the response to thiocyanate, with respect to the 24-hour uptake of radioiodine, appeared to differ quantitatively from that of the euthyroid subjects. This distinct difference in response could conceivably prove useful in distinguishing between toxic and nontoxic states.

The mechanism whereby thyrotropin induced iodide accumulation in the thiocyanate-inhibited euthyroid gland is not clear. It has been shown that the concentration of iodide in the thyroid gland may be increased by thyrotropin (7, 8) and, independently of thyrotropin, may be enhanced by a reduction in the intrathyroidal content of organic iodine (9, 10). In the present studies there was no clear indication as to whether thyrotropin achieved its effect directly by augmenting cellular "trapping" activity, or indirectly by lowering the intrathyroidal content of organic iodine, or by some other means.

The question arises whether thyrotropin may not also influence the intra-

TABLE 1. SUMMARY OF RESULTS IN EUTHYROID AND HYPERTHYROID SUBJECTS.

The baseline thyroidal radioiodine uptake for each subject (before administration of thiocyanate) is the first value in the last column—opposite the blank for the thiocyanate value. Repeat uptake values are shown opposite the corresponding serum thiocyanate levels, as well as in relation to administration of thryotropin.

Subjects	Sex	Serum SCN (mg./100 ml.)	Dosage of TSH	Thyroidal 24-hr. I^{131} uptake (% dose)
Euthyroid				
J.S.	M	—		36.0
		3.6		8.2
A.G.	M	—		16.0
		4.9		5.4
A.M.	F	—		48.0
		3.9		5.2
P.M.	M	—		32.0
		2.4		17.0
A.S.	M	—		37.4
		6.0		6.4
W.H.	M	—		25.6
		6.0		4.0
M.B.	F	—		34.4
		9.7		2.0
H.H.	F	—		31.4
		1.4		9.6
		5.2		6.9
		4.7	10 U×3 days	20.3
T.M.	M	—		34.5
		6.3		8.3
		7.0	10 U×3 days	20.1
A.K.	M	—		17.0
		5.0		1.5
		11.4	10 U×2 days	7.0
W.A.	M	—		23.2
		8.0		1.0
		7.8	20 U×1 day	8.0

TABLE 1. (*continued*)

Subject	Sex	Serum SCN (mg./100 ml.)	Dosage of TSH	Thyroidal 24-hr. I^{131} uptake (% dose)
E.T.	F	—		40.7
		4.8		6.9
		7.4		4.2
		11.0		2.0
		12.7	10 u×2 days	7.4
E.J.	F	—		47.8
		9.0		2.0
		11.6	20 u×1 day	7.4
M.R.	F	—		22.0
		10.0		2.0
		13.0	20 u×1 day	10.0
H.F.	M	—		17.0
		5.6		4.4
		7.5		3.2
		13.0	10 u×3 days	10.0
Hyperthyroid				
M.F.	F	—		68.0
		4.8		38.3
		9.0		23.4
		10.6	20 u×1 day	25.4
		13.0	10 u×3 days	19.5
B.N.	M	—		73.0
		6.0		49.5
		7.7		40.1
		8.8		32.8
		9.5	20 u×1 day	39.4
		11.4		24.1
D.B.	F	—		88.0
		6.5		53.5
		8.0		48.0
		10.5	30 u×1 day	34.0
		12.0		31.0

thyroidal metabolism of thiocyanate. No studies answer this question directly, but there are some data which disclose significant features in the metabolism of thiocyanate. It has been shown in the rat, using S^{35}-labeled thiocyanate, that a part of the thiocyanate becomes bound to protein and part is converted to water-soluble sulfur-containing compounds, the process being inhibited in the presence of propylthiouracil (11). Furthermore, thyroids made deficient in iodine by a low-iodine diet, or made hyperplastic by the administration of propylthiouracil, showed no greater concentration of thiocyanate than did the control thyroid glands (12). Thus, the indirect evidence is against the concept of a thyrotropin effect upon the thyroidal metabolism of thiocyanate.[5]

The failure of thyrotropin to augment thyroidal radioiodine uptake, seen in our thiocyanate-treated hyperthyroid patient, has also been observed in hyperthyroid subjects who had received no antithyroid medication (13). Whether these findings can be attributed to the same underlying mechanism remains to be elucidated. However, it has been shown that the concentrating capacity of the thyrotoxic gland is considerably increased (14). Thus, one might anticipate little or no response to thyrotropin if the thyroidal iodide-collecting mechanism were functioning maximally. Whether the inability of the thiocyanate ion to inhibit the activity of the hyperthyroid gland completely is a consequence of the hyperactive "trapping" mechanism, and not simply a phenomenon associated with glandular hypertrophy, must await further clarification.

REFERENCES

1. BARKER, M. H.: The blood cyanates in the treatment of hypertension, *J.A.M.A.* **106:** 762, 1936.
2. BARKER, M. H.; LINDBERG, H. A., and WALD, M. H.: Further experiences with thiocyanates; clinical and experimental observations, *J.A.M.A.* **117:** 1591, 1941.
3. ASTWOOD, E. B.: The chemical nature of compounds which inhibit the function of the thyroid gland, *J. Pharmacol. & Exper. Therap.* **78:** 79, 1943.
4. ASTWOOD, E. B.: Mechanisms of action of various antithyroid compounds, *Ann. New York Acad. Sc.* **50:** 419, 1949.
5. FRANKLIN, A. L.; CHAIKOFF, I. L., and LERNER, S. R.: Influence of goitrogenic substances on conversion in vitro of inorganic iodide to thyroxine and diiodotyrosine by thyroid tissue with radioactive iodine as indicator, *J. Biol. Chem.* **153:** 151, 1944.
6. FISTER, H. J.: Manual of Standardized Procedures for Spectrophotometric Chemistry. New York, Standard Scientific Supply Corp., 1950.
7. STANLEY, M. M., and ASTWOOD, E. B.: The response of the thyroid gland in normal human subjects to the administration of thyrotropin, as shown by studies with I^{131}, *Endocrinology* **44:** 49, 1949.

[5] Recent studies in this laboratory have shown that thyrotropin produces a twofold increase in thyroidal radioactivity compared with control values, when using S^{35}-labeled thiocyanate in guinea pigs.

8. VANDERLAAN, W. P., and GREER, M. A.: Some effects of the hypophysis on iodine metabolism by the thyroid gland of the rat, *Endocrinology* **47**: 36, 1950.
9. VANDERLAAN, W. P., and CAPLAN, R.: Observations on a relationship between total thyroid iodine content and the iodide-concentrating mechanism of the thyroid gland of the rat, *Endocrinology* **54**: 437, 1954.
10. HALMI, N. S., and SPIRTOS, B. N.: Analysis of the action of propylthiouracil on the pituitary-thyroid axis of rats, *Endocrinology* **55**: 613, 1954.
11. WOOD, J. L., and WILLIAMS, E. F., JR.: The metabolism of thiocyanate in the rat and its inhibition by propylthiouracil, *J. Biol. Chem.* **177**: 59, 1949.
12. WOOD, J. L., and KINGSLAND, N.: Labeled sulfur uptake by thyroids of rats with low plasma thiocyanate levels, *J. Biol. Chem.* **185**: 833, 1950.
13. MITCHELL, M. L.; BURROWS, B. A.; DELL, E. S., and GRAHAM, D. E.: The effect of thyroid-stimulating hormone on thyroidal iodine turnover, (abstract) *J. Clin. Endocrinol. & Metab.* **16**: 979, 1956.
14. STANLEY, M. M., and ASTWOOD, E. B.: The accumulation of radioactive iodide by the thyroid gland in normal and thyrotoxic subjects and the effect of thiocyanate on its discharge, *Endocrinology* **42**: 107, 1948.

IODIDE GOITER*

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ABSTRACT

Four women and 1 man receiving protracted treatment with iodides for asthma or bronchitis and exhibiting diffuse thyroidal enlargement were studied. Needle biopsy of the thyroid gland revealed parenchymatous hypertrophy in all. The goiter and myxedema receded on discontinuance of the use of iodides or on administration of thyroid. One patient, followed for more than two years, demonstrated rapid uptake of radioactive iodine over the thyroid gland when the serum iodide^{127} reached levels of 12.4 micrograms per 100 ml.; organic binding of iodine did not occur at that time, as documented by the lack of protein-bound radioiodine (PBI^{131}) in the serum (conversion ratio), the rapid decay of radioactivity from the thyroid gland at rates parallel with the decay from the thigh, and the appearance of radioactivity in the urine. Lack of organic binding also was demonstrated by historadiologic technics. When concentrations of iodide^{127} had reached 0.56 microgram per 100 ml. of serum, the uptake of I^{131} over the thyroid gland was avid and protracted; at that time, PBI^{131} appeared in the serum at a rate that was faster than normal. Studies with potassium iodide showed pronounced sensitivity of the thyroid in these patients to the inhibiting effects of iodides on the organic binding of iodine, quite comparable in nature to the findings in Graves' disease. The similarities between the clinical picture of iodide goiter and that of Hashimoto's thyroiditis are stressed.

ALMOST unusual complication of the protracted use of iodides is the development of a goiter, with or without myxedema. Less than a score of such instances have been reported in the English literature. The rarity of this syndrome is stressed by the contrastingly large number of patients who take iodides for asthma and other diseases without ever exhibiting any thyroidal abnormalities.

Myxedema following the administration of iodides was first reported by Haines (1) in a young woman who had postoperative recurrence of Graves' disease. Thompson and associates (2) and Proger and Brauns (3) described similar cases, and Hurxthal (4), Bell (5), Nixon (6) and others noted the

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occurrence of myxedema after the administration of iodides to patients who had pre-existing nontoxic goiters.

Because the cases to be reported concern patients who had no known abnormalities of the thyroid gland when treatment with iodides was begun, the literature that describes similar situations warrants specific recounting. Morgans and Trotter (7) reported 2 such cases in 1953; they were the first to demonstrate a high uptake of radioiodine by the thyroid shortly after the cessation of therapy with iodides. Turner and Howard (8) added 13 cases, all of which were in children; 7 of their patients exhibited myxedema in addition to the goiter. Rubenstein and Oliner (9) recently reported the case of a 33-year-old woman in whom first a goiter and later myxedema developed after four years of daily intake of iodides for bronchial asthma. One day after the use of iodides was stopped, the two-hour uptake of I^{131} over the thyroid was 70 per cent of the administered dose and the thyroidal radioactivity decayed rapidly. Concomitant failure of organic binding was demonstrated by the lack of production of radioactive protein-bound iodine (PBI^{131}). Seven days after the use of iodides was stopped, the thyroidal uptake of I^{131} was high and remained increased for a protracted period; it did not decrease with the administration of potassium thiocyanate. At that time, PBI^{131} was being rapidly produced. Paley and associates (10) studied a 49-year-old woman who had iodide myxedema and goiter documented by needle biopsy of the thyroid. When the concentration of serum iodide exceeded 300 micrograms per 100 ml., organic binding of iodide by the thyroid did not occur. At later stages, when the value for serum iodide was 2 micrograms per 100 ml., organic binding was shown to proceed at a fast rate.

The case reported by Raben (11) probably constitutes an example of iodide goiter and myxedema, even though the only apparent exposure to iodides was in the form of bronchography carried out nine months before the appearance of the goiter. Biopsy material was not available for diagnostic confirmation. However, other diagnoses were unlikely because of the early high uptake of I^{131} over the thyroid gland, the urinary iodide excretion of 1173 micrograms per twenty-four hours, the serum PBI level of 9.61 micrograms per 100 ml., and the failure of the goiter and myxedema to reappear after discontinuance of treatment with desiccated thyroid.

Our observations corroborate and expand those of others, suggesting that, in the comparatively small group of persons in whom goiter develops after administration of iodides, the organic binding of iodine within the thyroid gland may be unusually susceptible to the inhibitory effects of iodides; moreover, a similarity in response to the administration of iodides in patients with iodide goiter and those with exophthalmic goiter was ob-

served, together with several clinical features common to iodide goiter and Hashimoto's thyroiditis.

MATERIAL AND METHODS

The records of 11 cases diagnosed as iodide goiter at the Mayo Clinic since 1956 were reviewed. Eight of the patients were adults and 3 were children; 9 were female and 2 were male. The difficulties encountered in documenting the diagnosis of iodide goiter or myxedema or both made us limit the present report to 5 cases in which biopsy material from the thyroid was available for study and the histologic picture was that of parenchymatous hypertrophy.[1] Determinations of the basal metabolic rate, and serum PBI and butanol-extractable iodine (BEI) levels, as well as I^{131} tracer data were available in the majority of cases. Studies of I^{131} clearance were performed on 2 patients by the technic of Berson. The same 2 patients were given carrier doses of 2 mg. of iodine as potassium iodide, and the magnitude of the inhibitory effects of iodides on the thyroidal uptake of I^{131} was observed in both. Historadiologic studies on thyroidal organic binding of iodine were performed on one of these patients.

RESULTS

The essential findings in 4 of our 5 cases of iodide goiter are summarized in Table 1. Common to all cases was parenchymatous hypertrophy of the thyroid on biopsy and the intake of iodides for uninterrupted and prolonged periods ranging from thirteen months to more than seven years. A diffuse firm goiter was present in all but Case 4, in which the thyroid was soft, with a loud bruit over both lobes. Three of these patients were women taking iodides for asthma; the fourth was a man who had chronic bronchitis. Three patients were myxedematous and 1 was euthyroid. The basal metabolic rates ranged from -5 to -22 per cent. The serum PBI level, when measured, usually was increased; the BEI level was low in 1 patient. Tracer studies with I^{131} were performed on all of these patients at intervals of six and twenty-four hours after the administration of the I^{131}. The uptake by the thyroid, when enough time had elapsed from the last dose of iodide-containing medicaments, was uniformly increased. In the majority of instances, it was impossible to interrupt the administration of iodide-containing medicaments, but the goiter and the manifestations of myxedema disappeared during treatment with desiccated thyroid. In Case 4, use of iodides was permanently discontinued and treatment with thyroid was

[1] The clinical course of the disorder in 2 children made the diagnosis of iodide goiter a highly probable one. These were 5-year-old twin sisters receiving iodides for bronchial asthma. The smallness of their goiters precluded needle biopsy. It was possible to withhold iodides from one twin at a time, while the sister continued to take iodides as a control. This procedure was reversed for two full cycles. When iodides were withheld, the goiter disappeared within four weeks, reappearing within a comparable period when iodides were readministered. Ultimately, the use of iodides was discontinued in both patients, and their thyroids reverted to normal size and consistency.

not instituted; complete resolution of the myxedema and the goiter was seen within three months. Case 3 is especially noteworthy, as the preoperative diagnosis was Hashimoto's thyroiditis; to the surprise of both surgeon and clinician, the histologic appearance of the thyroid was that of parenchymatous hypertrophy.

The fifth of our patients (Case 5) was observed for a period of two years. The essential data[2] in this case are summarized in Table 2. This 33-year-

TABLE 1. ESSENTIAL FINDINGS IN 4 PATIENTS WITH IODIDE GOITER

Patient	Age (yrs.), sex, clinical status	BMR (%)	Blood iodides (μg./100 ml.)		I^{131} tracer studies			Treatment and follow-up
			PBI	BEI	Days without iodides	24-hr. *in vivo* (%)	Clearance (ml./min.)	
1	27 F Asthma; myxedema	−16	14.4	8.1	0 4	2 —	— 154	Desiccated thyroid; myxedema disappeared; goiter receded
2	59 F Asthma; myxedema	—	13.0	2.3	12	82	—	Desiccated thyroid; no follow-up
3	47 F Asthma; euthyroid	− 5	—	—	10	83	—	Thyroidectomy; desiccated thyroid; no recurrence
4*	54 M Myxedema	−22	—	—	60†	43	214	Iodides withdrawn; myxedema and goiter disappeared

* Soft, mushy thyroid with loud bruit.

† Continued to receive 150 micrograms of potassium iodide daily until day of tracer. Larger doses discontinued 60 days before.

old woman had taken potassium iodide for thirteen months because of asthma. When first examined, in 1956, she appeared euthyroid. The thyroid was diffusely enlarged to approximately three times normal size, and was firm. Needle biopsy revealed parenchymatous thyroidal hypertrophy (Fig. 1). Nine days after the first withdrawal of iodides, the thyroidal uptake of I^{131} was 92 per cent at twenty-four hours. The conversion ratio at forty-eight hours was 100 per cent, and the serum PBI^{131} was shown by chromatography to be thyroxine. The goiter disappeared concomitantly with the administration of desiccated thyroid and failed to reappear when use of iodides was reinstituted one year later. In 1958, in an effort to reproduce the goiter, the patient agreed to discontinue only the use of desiccated

[2] The values for PBI and BEI were invalidated by cholecystography performed in May 1955. When the patient was first examined, the value for BEI was 4000 micrograms per 100 ml. of serum; it decreased steadily to 1800 micrograms on August 25, 1958. It is noteworthy that this high concentration of organically bound iodine did not interfere with the performance of thyroidal function tests with radioisotopes.

thyroid; a goiter and mild myxedema became apparent within five weeks. Use of iodides then was stopped; twenty-four hours later, the serum inorganic iodide measured 12.4 micrograms per 100 ml. and the thyroidal clearance of I^{131} was 74 ml. per minute, with a peak uptake of 23.8 per cent at fifty minutes and a steady decrease thereafter. The conversion ratio at twenty-four hours was 0 per cent.

Figure 2 shows the uptake of radioactive iodine by the thyroid gland ex-

TABLE 2. IODIDE GOITER IN A 33-YEAR-OLD ASTHMATIC WOMAN: POTASSIUM IODIDE FOR THIRTEEN MONTHS (CASE 5)

		Oct. 20, 1956	Feb. 11, 1958	July 7, 1958	Aug. 25, 1958	Sept. 5, 1958
Thyroid gland		3× normal, diffuse, firm*	Normal	Normal	2× normal, soft, diffuse; bruit	2× normal, soft, diffuse
Clinical status		Euthyroid	Euthyroid	Euthyroid	Mild myxedema	Euthyroid
BMR (%)		−11	—	—	−18	—
Inorganic iodide of serum (μg./100 ml.)		—	—	—	12.4	0.56
I^{131} thyroidal uptake (%)	6-hr.	84	27	—	17	55
	24-hr.	92	38	—	10	65
I^{131} clearance (ml./min.)		—	—	—	74	85
PBI^{131} of serum (% of dose per liter)		0.310†	—	—	0	0.119‡
Iodide medication		None for 9 days	None	KI from Feb. 15 to Aug. 24, 1958	None for 1 day	None for 12 days
Desiccated thyroid		None	60 mg. per day since Oct. 30, 1956	90 mg. per day since Feb. 14, 1958	None for 49 days	None for 61 days

* Parenchymatous hypertrophy on biopsy. Diffuse I^{131} uptake on gammagram.
† Conversion ratio = 100 per cent at 48 hours.
‡ Conversion ratio = 70 per cent at 22 hours.

pressed as the percentage uptake of the dose when use of iodides had been discontinued for twenty-two hours. The initial rapid uptake was followed by a steady slow decay of radioactivity; the rate of this decay was paralleled by the disappearance rate over the thigh (Fig. 3). The appearance of radioactivity in the urine occurred at a rate of 6.2 per cent of the dose per hour, which approximated closely the decay values over the thyroid (6.6 per cent per hour) and thigh (7.1 per cent per hour). Similar studies were repeated forty-eight hours after withdrawal of iodides, with almost identical findings. Results of radioautographic studies on biopsy material obtained from the thyroid at that time are shown in Figure 4. The tissue was

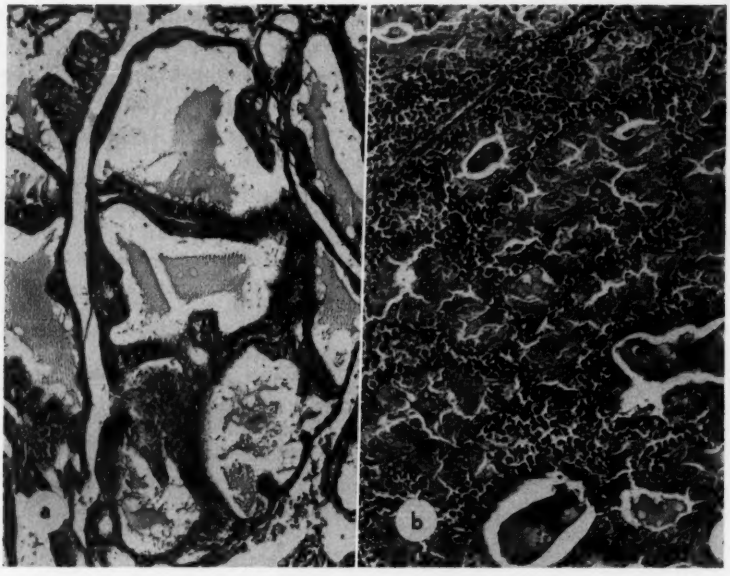

FIG. 1. **a.** Thyroid in Case 5, showing parenchymatous hypertrophy in iodide goiter. Sample obtained by needle biopsy on October 26, 1956 (hematoxylin and eosin; ×100). **b.** Typical appearance in Hashimoto's thyroiditis for contrast (hematoxylin and eosin; ×150).

frozen immediately in isopentane cooled with liquid nitrogen. The frozen tissue was dehydrated in a vacuum (less than 10^{-3} mm. of mercury) at −30° C. in the manner described by Stowell (12). Radioautographs were

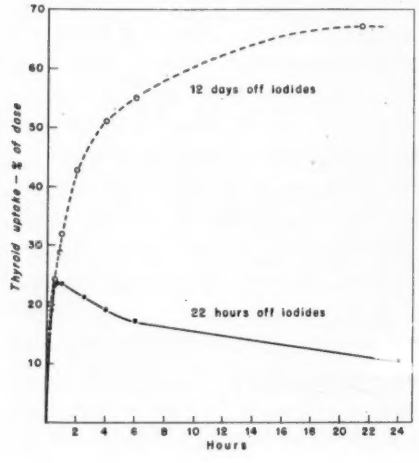

FIG. 2 (Case 5). Thyroidal uptake of I^{131} twenty-two hours after withdrawal of iodides, when serum inorganic iodide level was 12.4 micrograms per 100 ml., and twelve days later, when serum inorganic iodide level had decreased to 0.56 microgram per 100 ml.

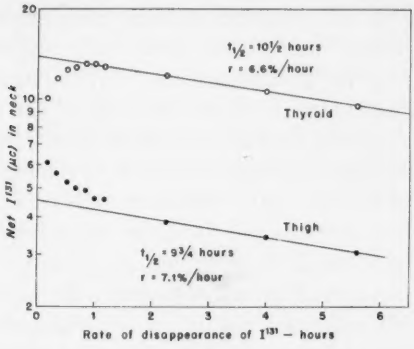

FIG. 3 (Case 5). Rate of disappearance of I^{131} from the thyroid and thigh twenty-two hours after withdrawal of iodides.

prepared from this tissue by mounting paraffin-embedded microtome sections directly on glass slides (Fig. 4*a*). Additional freeze-dried paraffin sections were treated with distilled water, with a 1 per cent solution of trichloroacetic acid and with a 1 per cent solution of silver nitrate prior to preparation of the radioautographs. The radioactivity was removed almost completely by the action of distilled water (Fig. 4*b*). A small amount of activity remained following extraction by trichloroacetic acid (Fig. 4*c*), and the tissues treated with silver nitrate possessed radioactivity comparable to that of untreated sections (Fig. 4*d*).

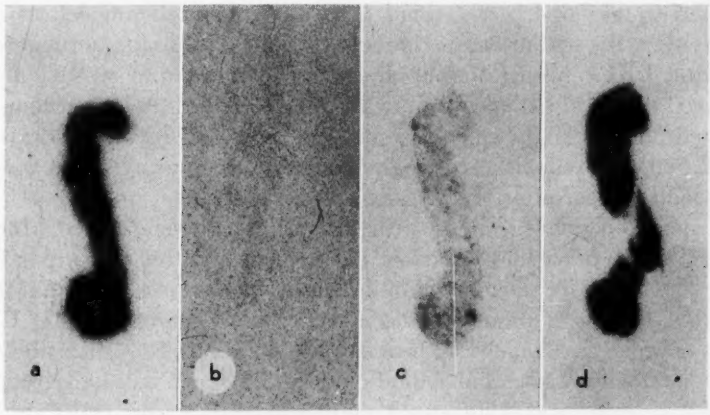

FIG. 4. (Case 5). Radioautographs prepared from serial sections of tissue obtained at biopsy of iodide goiter (freeze-dried tissue; ×6). **a.** Total radioactivity in section mounted directly from microtome without exposure to any reagent. **b.** Most of radioactivity was removed from section washed with distilled water. **c.** Small amount of radioactivity was retained in section placed in a 1 per cent solution of trichloroacetic acid. **d.** Amount of radioactivity retained in section placed in a 1 per cent solution of silver nitrate is comparable to total radioactivity (see **a**).

It was concluded from these studies that the major radioactive component was iodide, which was soluble in water and trichloroacetic acid and was precipitated by silver nitrate. The small amount of radioactivity demonstrable after treatment of the tissues with trichloroacetic acid probably was bound to protein. Two weeks later, the concentration of serum iodide^{127} had decreased to 0.56 microgram per 100 ml. The thyroidal uptake of I^{131} at twenty-two hours was 64.5 per cent and, in contrast to the situation two weeks before, the value for PBI^{131} at twenty-four hours was 0.119 per cent of the dose per liter of serum, reflected by a conversion ratio of 70 per cent. Figure 2 also shows the thyroidal uptake of I^{131} twelve days after withdrawal of iodides, indicating a steady and prolonged increase in uptake without the rapid discharge of radioactivity observed two weeks previously.

COMMENT

In the differential diagnosis of an iodide goiter, Hashimoto's thyroiditis always should be considered. The case reported by Hydovitz and Rose (13) illustrates this point. It concerned a 52-year-old woman under protracted iodide therapy for bronchial asthma whose goiter and myxedema were thought to be caused by the iodides. No biopsy material was available to substantiate the diagnosis. However, factors favoring the diagnosis of Hashimoto's thyroiditis with concomitant use of iodides included 1) a high thyroidal uptake of I^{131} and rapid decay of thyroidal radioactivity two months after the withdrawal of iodides, 2) normal and high normal values for serum PBI concentration attended by low amounts of BEI after a prolonged period of iodide withdrawal, and 3) the fact that myxedema disappeared only partially and the goiter not at all after the withdrawal of iodides, with myxedema recurring when use of triiodothyronine was discontinued one year after the last dose of iodides.

The possibility of Hashimoto's thyroiditis was entertained by Morgans and Trotter (7) in one of their cases, but the goiter in this instance had disappeared entirely on withdrawal of iodides without the concomitant use of thyroxine, making it almost a certainty that the diagnosis was not Hashimoto's thyroiditis. Table 3 summarizes the differential diagnosis between Hashimoto's thyroiditis and iodide goiter. The gland is usually diffusely enlarged in both conditions, and it may be either firm or soft. The metabolic status may be that of euthyroidism or myxedema. The basal metabolic rate may be normal or low in both conditions. The value for serum PBI is usually low in Hashimoto's thyroiditis, but it can be normal or, on occasion, high; it is usually high in iodide goiter when the patient is receiving iodides. The amount of serum BEI is usually low in both conditions. The uptake of I^{131} by the thyroid may be high, normal or low in

Hashimoto's thyroiditis, whereas it is usually high in iodide goiter when use of the iodide-containing medicament has been interrupted for a sufficiently long time prior to the test. Not shown in Table 3, but of importance, is the fact that the goiter and the hypothyroidism in both iodide goiter and Hashimoto's thyroiditis usually disappear during administration of desiccated thyroid. The histologic appearance of the goiter, on the other hand, is entirely different in the two conditions (Fig. 1). Iodide

TABLE 3. DIFFERENTIAL DIAGNOSIS BETWEEN HASHIMOTO'S THYROIDITIS AND IODIDE GOITER

	Hashimoto's thyroiditis	Iodide goiter
Goiter	Diffuse or lobulated; firm or soft	Diffuse; firm or soft
Metabolic status	Euthyroid or myxedema	Euthyroid or myxedema
BMR	Low or normal	Low or normal
Serum PBI	Low or normal	High when on iodides
Serum BEI	Low or normal	Usually low
Thyroidal I^{131} uptake	Normal or high	High when no iodides administered
Histologic features	Enlargement and oxyphilia of epithelial cells; loss of colloid; infiltration with plasma cells and lymphocytes; formation of germinal centers; fibrosis	Parenchymatous hypertrophy; variable amounts of colloid storage

goiters are hyperplastic, which appears to be an important contribution to accurate diagnosis in these cases. The diagnosis of iodide goiter was confirmed on biopsy in the case of Paley's group (10), one of Bell's (5) cases and one of the cases of Turner and Howard (8).

A rather striking characteristic of iodide goiters is the way in which their uptake of I^{131} is inhibited by small amounts of iodide. Three of our patients were studied before and after the administration of 2 mg. of iodine as potassium iodide in the manner described by Feinberg and associates (14). Table 4 shows the results of the studies in these patients, along with comparable observations obtained in euthyroid patients and in patients who had Graves' disease. The similarity of response between the hyperthyroid patients and those with iodide goiter is obvious. The literature contains many accounts of the relationship between iodide myxedema and

exophthalmic goiter; in fact, the early descriptions of iodide myxedema occurred in patients with postoperative Graves' disease who were receiving iodides. In the case described by VanderLaan (15) an iodide goiter preceded the appearance of Graves' disease.

Iodide goiters are probably mediated by thyroid-stimulating hormone (TSH). Excessive amounts of iodide have been shown to inhibit organifica-

TABLE 4. INHIBITORY EFFECTS OF IODINE ON THYROIDAL UPTAKE OF I^{131}

Condition		Thyroidal I^{131} uptake (% of dose)			
		Control		After 2 mg. of iodine as KI	
		6-hr.	24-hr.	6-hr.	24-hr.
Euthyroid (average values in 12 patients*)		21	35	11	22
Graves' disease (average values in 4 untreated patients*)		69	70	14	16
Iodide goiters	Case 2: 12 days after withdrawal of iodides	76	82	36	24
	Case 4: 5 months after withdrawal of iodides	57	68	19	13
	Case 5: receiving 60 mg. of desiccated thyroid daily; no iodides for 16 months	27	38	6	12
	Case 5: no iodides for 13 days; no thyroid for 2 months	55	65	10	5

* From Feinberg and associates (14).

tion of iodine in humans and laboratory animals (16–18). The resulting increase of TSH output produces enlargement of the thyroid and also an augmentation of the "trapped" iodide. This increase in the trapping mechanism explains the early uptake of I^{131} over the thyroid of the patient with iodide goiter, even at a time when the concentration of plasma iodide^{127} is high. The available data suggest that lesser amounts of iodide are necessary to inhibit organic binding of iodine in patients with iodide goiter than are required in normal persons. It is also apparent that patients with iodide goiter do not "escape" from the inhibitory effects of iodides, whereas normal persons do.

REFERENCES

1. HAINES, S. F.: Exophthalmic goiter and myxedema: report of a case, *Endocrinology* **12:** 55 (Jan.–Feb.) 1928.
2. THOMPSON, W. O.; THOMPSON, P. K.; BRAILEY, A. G., and COHEN, A. C.: Myxedema during the administration of iodin in exophthalmic goiter, *Am. J. M. Sc.* **179:** 733 (June) 1930.
3. PROGER, S., and BRAUNS, W. H.: Myxedema following iodine treatment for hyperthyroidism, *Bull. New England M. Center* **5:** 42 (Feb.) 1943.
4. HURXTHAL, L. M.: Myxedema following iodine administration for goiter in a girl aged six years, *Lahey Clin. Bull.* **4:** 73 (Jan.) 1945.
5. BELL, G. O.: Prolonged administration of iodide in the pathogenesis of simple goiter and myxedema, *Tr. Am. Goiter A.*, pp. 28–37, 1952.
6. NIXON, P. G. F.: Recurrent myxoedema goitre attributed to potassium iodide, *Brit. M. J.* **1:** 748 (Mar. 30) 1957.
7. MORGANS, M. E., and TROTTER, W. R.: Two cases of myxoedema attributed to iodine administration, *Lancet* **2:** 1335 (Dec. 26) 1953.
8. TURNER, H. H., and HOWARD, R. B.: Goiter from prolonged ingestion of iodide, *J. Clin. Endocrinol. & Metab.* **16:** 141 (Jan.) 1956.
9. RUBENSTEIN, H. M., and OLINER, L.: Myxedema induced by prolonged iodide administration, *New England J. Med.* **256:** 47 (Jan. 10) 1957.
10. PALEY, K. R.; SOBEL, E. S., and YALOW, R. S.: Some aspects of thyroidal iodine metabolism in a case of iodine-induced hypothyroidism, *J. Clin. Endocrinol. & Metab.* **18:** 79 (Jan.) 1958.
11. RABEN, M. S.: Endocrine conference, *J. Clin. Endocrinol. & Metab.* **13:** 469 (Apr.) 1953.
12. STOWELL, R. E.: A modified freezing-drying apparatus for tissues, *Stain Technol.* **26:** 105 (Apr.) 1951.
13. HYDOVITZ, J. D., and ROSE, E.: Goiter and myxedema following prolonged ingestion of iodine: report of a case with evidence of unusual thyroid dysfunction, *J. Clin. Endocrinol. & Metab.* **16:** 1109 (Aug.) 1956.
14. FEINBERG, W. D.; HOFFMAN, D. L., and OWEN, C. A., JR.: The effects of varying amounts of stable iodide on the function of the human thyroid, *J. Clin. Endocrinol. & Metab.* **19:** 567 (May) 1959.
15. VANDERLAAN, W. P.: Myxedema and goiter attributed to iodine ingestion in a patient subsequently developing hyperthyroidism, *Metabolism* (Pt. 1) **5:** 640 (Nov.) 1956.
16. WOLFF, J., and CHAIKOFF, I. L.: The inhibitory action of excessive iodide upon the synthesis of diiodotyrosine and of thyroxine in thyroid gland of the normal rat, *Endocrinology* **43:** 174 (Sept.) 1948.
17. STANLEY, M. M.: The direct estimation of the rate of thyroid hormone formation in man. The effect of the iodide ion on thyroid iodine utilization, *J. Clin. Endocrinol.* **9:** 941 (Oct.) 1949.
18. CHILDS, D. S., JR.; KEATING, F. R., JR.; RALL, J. E.; WILLIAMS, M. M. D., and POWER, M. H.: Effect of varying quantities of inorganic iodide (carrier) on urinary excretion and thyroidal accumulation of radioiodine in exophthalmic goiter, *J. Clin Invest.* **29:** 726 (June) 1950.

THE METABOLISM OF TRIIODOTHYRONINE IN GRAVES' DISEASE*†

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ABSTRACT

Chromatographically pure triiodothyronine (T_3) labeled with I^{131} was given to 12 patients with current or recent hyperthyroidism of Graves' disease (4 still hyperthyroid, 5 euthyroid, and 3 hypothyroid) and to 8 normal controls. The fate of T_3 in these patients was observed by quantitative chromatography carried out on serial samples of blood and urine. In addition, radioactivity over the liver was measured. The behavior of the injected T_3 in the patients with Graves' disease, regardless of their metabolic state, was quite different from that in the controls. During the first forty-eight hours, the labeled T_3 remained in the serum of patients with Graves' disease in greater amounts than in the controls. More radioactivity was excreted in the urine by patients with Graves' disease than by the controls; this difference was largely owing to free iodide. There was also slightly more unaltered T_3 in the urine of patients with Graves' disease. Measurement of radioactivity over the liver revealed maximal concentration within a few minutes after injection of T_3, followed by a marked fall. A subsequent secondary rise occurred in the patients with Graves' disease, but not in the controls. The liver/serum-T_3 ratio indicated that, considering the level of T_3 in serum, the livers of the patients with Graves' disease held less radioactivity than did the livers of the normal subjects.

SINCE the identification of triiodothyronine in human serum by Gross and Pitt-Rivers (1), the role of this compound has been investigated *in vivo* and *in vitro*. Its calorigenic effect is greater and of shorter duration than that of thyroxine (2–6). Although triiodothyronine has been found in in the circulation of patients with thyrotoxicosis and some normal subjects (7–9), the amounts have been very small; however, they might be extremely significant. It has been postulated by some (10, 11) that thyroxine might be a precursor of triiodothyronine, and by others (8, 12) that triiodothyronine might arise as a primary secretion from the thyroid. Studies conducted after the administration of radioactive iodine have shown that in some cases of thyroid disease triiodothyronine appears in the serum be-

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fore, and initially in larger amounts than thyroxine, but as time progresses, thyroxine becomes the dominant iodinated compound of the serum (9). What part, if any, triiodothyronine may play in Graves' disease is not clear, but at least it may be said that triiodothyronine is most readily detectable in the serum of patients with the most severe degrees of thyrotoxicosis (9).

Triiodothyronine has been shown to accumulate in large quantities in the liver of rats, followed by rapid clearance of the radioactivity (13), in association with the appearance of conjugated iodinated compounds in the bile (14–16). When the liver was removed or the common bile duct ligated, the conjugated triiodothyronine in the blood and urine increased (17, 18), suggesting further that the liver normally plays a major role in the metabolism of triiodothyronine.

Because triiodothyronine might play an important part in thyrotoxicosis, it seemed appropriate to explore the fate of this compound when it was injected into patients with Graves' disease. Simultaneous observations were made on changes of radioactivity in the serum, urine, and in the area of the liver.

METHODS

Patients with proven Graves' disease,[1] either current or recent, were selected for this investigation. Some of them had received definitive therapy which had resulted in a euthyroid or hypothyroid state. Great care was taken to select only patients who fell clearly into one of the following three groups: 1) hyperthyroid, 2) euthyroid, or 3) hypothyroid. One of the authors had originally observed all but one[1] of the patients while the classic signs of hyperthyroidism were present and before any therapy had been given. The original diagnosis and the diagnosis of the clinical state at the time of this study were based on the clinical history, physical examination, uptake of radioiodine by the thyroid, basal metabolic rate, and in some instances the serum protein-bound iodine determination.

Twenty patients were studied. Twelve were in the Graves' disease group; 4 were hyperthyroid, 5 were euthyroid, and 3 were hypothyroid (2 with frank myxedema) at the time of the study. Eight control subjects (known to be free from renal disease, liver disease, and cardiac failure) were selected from volunteers and from patients who had been hospitalized for incidental lesions entirely unrelated to the thyroid.

A tracer dose of I^{131}-labeled 3,5,3′-triiodothyronine[2] (not exceeding 200 microcuries) was injected into the antecubital vein of the subject to be studied. The specific activity of the material injected ranged from 30 to 50 microcuries per microgram of iodine. Before administration each sample of triiodothyronine was found by chromatography (butanol:dioxane:ammonia system) to have no less than 97 per cent of the radioactivity in the form of triiodothyronine, the only radioactive contaminant being iodide. To insure against the thyroid playing a part in the formation and secretion of labeled iodinated compounds, a dose of 100 mg. of propylthiouracil was given to all subjects

[1] When first seen, one of the patients had myxedema. There was no preceding history which definitely suggested hyperthyroidism.

[2] Reported by Abbott Laboratories, the manufacturer, to be labeled in the 3′ position.

twelve hours before the injection of triiodothyronine, and repeated every six hours for the duration of the study.

Observations were made on the blood, urine, liver and thyroid at intervals during the seventy-two hours following the injection of triiodothyronine. Samples of blood were drawn at 1, 2, 4, 8, 24, 48 and 72 hours following the injection. A volume of 3 ml. of serum was counted in a well-type scintillation counter, and extracted four times with n-butanol. The nonextractable residue was redissolved in 6*N* sodium hydroxide and counted to determine the extractability of the labeled iodinated compounds. The butanol extract was dried under vacuum, redissolved in 0.3 ml. of n-butanol, and chromatographed in a descending butanol:dioxane:ammonia system together with unlabeled carriers of iodide, triiodothyronine, and thyroxine. The resulting strip chromatograms were cut into segments $\frac{1}{4}$-inch wide and counted on an automatic sample changer with an end-window beta counter (12). The amounts of radioactivity in the individual compounds of the chromatograms were then calculated and related to the administered dose of labeled triiodothyronine.

All urine was collected in separate samples coinciding with the sampling of blood. The radioactivity of an aliquot of each sample of urine was determined and related to the original volume. A known volume of urine, depending on its content of radioactivity, was measured and poured onto a prepared column of Dowex 1 resin and eluted with glacial acetic acid. All inorganic iodide was retained in the column while the iodinated organic compounds were eluted.[3] The total radioactivity in the eluate was determined and quantitative chromatograms of the iodinated organic compounds in these eluates were made by the same method as that used on the serum. All samples of eluate from the first 13 patients and random samples from the remaining patients were chromatographed, representing more than 100 samples. Thus, the radioactivity of all fractions, as well as the total excreted, was related to that of the injected triiodothyronine.

An estimation of the amount of radioactivity in the region of the liver was made, using a shielded scintillation counter. Various corrections for body background were applied. Crude periodic estimates of radioactivity over the liver in the first few patients revealed unexpected findings which prompted refinements in the methods. Improved geometry and continuous recording during the first eight hours in subsequent patients of the study served to document more fully the changes which occurred. The effective field of the shielded counter was arranged with fixed geometry over areas drawn on the right and left sides of the abdomen. The position on the right side corresponded with the largest cross-sectional area of the liver. Counting proceeded alternately from these two fixed positions and was continuously recorded with an Esterline-Angus recorder. During the counting procedure, lead shielding was placed over the opposite side of the abdomen (to the mid line) from that which was being counted. This was done to enhance the significance of the difference in radioactivity detected on the respective sides. This difference represented the number of counts attributable to the liver. Subsequent observations with the same fixed geometry were made at 24, 48 and 72 hours. The livers of 11 patients were surveyed in this manner, and data procured only by this method have been used in Figure 4. It was found that by using these procedures it was possible to estimate within 5 per cent the amount of radioactivity within a mock liver in a dummy.

From time to time during the period of observations, measurement of radioactivity

[3] As described elsewhere, this method resulted in a recovery of 90 per cent triiodathyronine, 94 per cent thyroxine, 92 per cent monoiodotyrosine and 93 per cent diiodotyrosine, whereas only 0.28 per cent of iodide was eluted from the column (19).

over the thyroid was carried out and releated to that in other areas of the body to insure that the thyroid was not playing an active part in the secretion of labeled compounds.

RESULTS

There was remarkable similarity in the total urinary excretion of I^{131} among the various groups with Graves' disease. Regardless of the metabolic state, the amounts were far greater than in the controls. The average total amount of I^{131} excreted over seventy-two hours in the Graves' disease groups was 71.3 ± 11.4 per cent of the dose. In contrast, the controls excreted an average of only 45.8 ± 14.5 per cent of the dose in seventy-two hours, the range being 57.7 to 30.1. The difference between patients with Graves' disease and normal subjects was significant ($P < .005$). Among those with Graves' disease, the range for the hyperthyroid group was 75.4 to 66.9 per cent, for the euthyroid group 82.0 to 43.2 per cent, and for the hypothyroid group 83.8 to 68.8 per cent of the dose. Thus, for this period of time, patients with Graves' disease, whether toxic or not, excreted in the urine more radioactivity from a dose of labeled triiodothyronine than did normal subjects.

It was found by chromatography that the radioactivity in the urine was only in the form of free iodide and unaltered triiodothyronine.[4] No other compounds were detected in the urine of either patients with Graves' disease or normal controls, irrespective of the elapsed time following the injection of triiodothyronine.

The increased amount of radioactivity excreted in the urine of patients with Graves' disease was shown by chromatography to be attributable to increased amounts of iodide. In the 72-hour period following the injection of triiodothyronine, the patients with Graves' disease had excreted 68.1 ± 11.9 per cent of the administered radioactivity as inorganic iodide, whereas the controls had excreted only 44.6 ± 9.0 per cent. There was only 1 patient with Graves' disease in whom the excretion of I^{131} iodide was as low as the highest control value (Fig. 1).

The total excretion of unaltered triiodothyronine in the urine in seventy-two hours by the patients with Graves' disease was 3.16 ± 0.42 per cent of the dose of triiodothyronine, whereas that of controls was only 1.33 ± 0.33 per cent (significance, $P < .005$). Among the patients with Graves' disease, those that were hyperthyroid excreted 3.72 ± 0.37 per cent, those that were euthyroid excreted 3.02 ± 0.43 per cent, and those that were hypothyroid excreted 2.79 ± 0.23 per cent. The difference between the hyperthyroid and the hypothyroid groups is probably significant ($P < .01$). By the time

[4] With single-phase chromatography, the presence of trace amounts of labeled iodinated acetic and pyruvic acid derivatives of thyronine cannot be completely excluded in the presence of the relatively large amounts of triiodothyronine.

twenty-four hours had passed, the cumulative excretion of unaltered triiodothyronine in all patients with Graves' disease was greater than that in any of the controls (Fig. 2).

Interesting differences were found in the amounts of labeled triiodothyronine remaining in the serum of both patients with Graves' disease and normal subjects. One hour after the administration of triiodothyronine the amount of this substance remaining unaltered in the serum of patients with Graves' disease was 10.0 ± 4.7 per cent of the dose per liter of serum, whereas in the controls it was 5.8 ± 2.6 per cent (Table 1). At this time, in

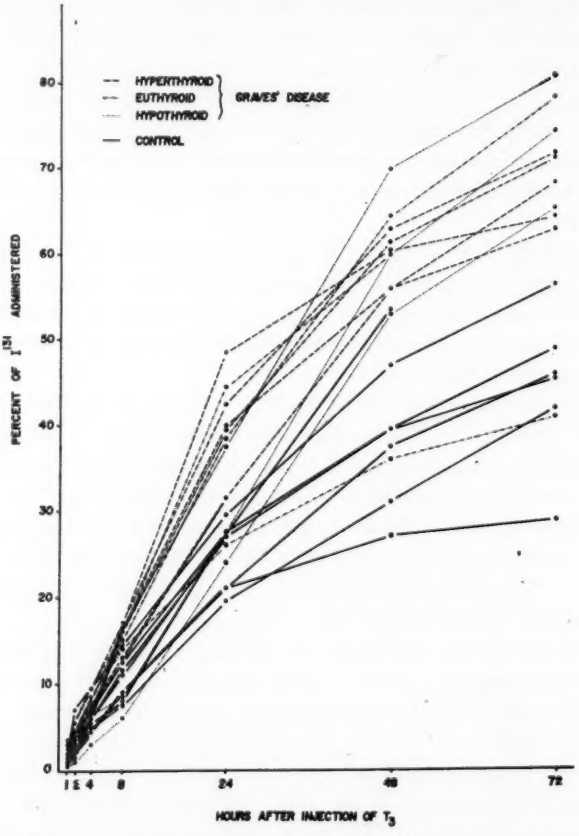

FIG. 1. Cumulative excretion of I^{131} iodide in the urine, expressed as a percentage of the injected radioactivity. The difference between patients with Graves' disease and the normal controls became evident after eight hours. Only 1 patient with Graves' disease, rendered euthyroid by the administration of propylthiouracil for forty weeks, exhibited a pattern of excretion of iodide like that of normal subjects.

the Graves' disease group, iodide represented 2.3 ± 0.8 per cent of the dose per liter of serum, and in the controls iodide represented 1.5 ± 0.5 per cent.

When the one-hour level of triiodothyronine in the serum was expressed as 100 per cent and subsequent values were expressed as a percentage of that initial value, it was observed that in most of the patients with Graves' disease there was a delay in the decline of triiodothyronine in the serum when compared to the controls. At the end of the first eight hours there was 22 ± 1.4 per cent of the first-hour level remaining in the controls, and there was 33 ± 7.7 per cent in the patients with Graves' disease. At this time only 1 of the patients with Graves' disease showed a level which was as low as the highest value among the controls. Thus the initial levels in

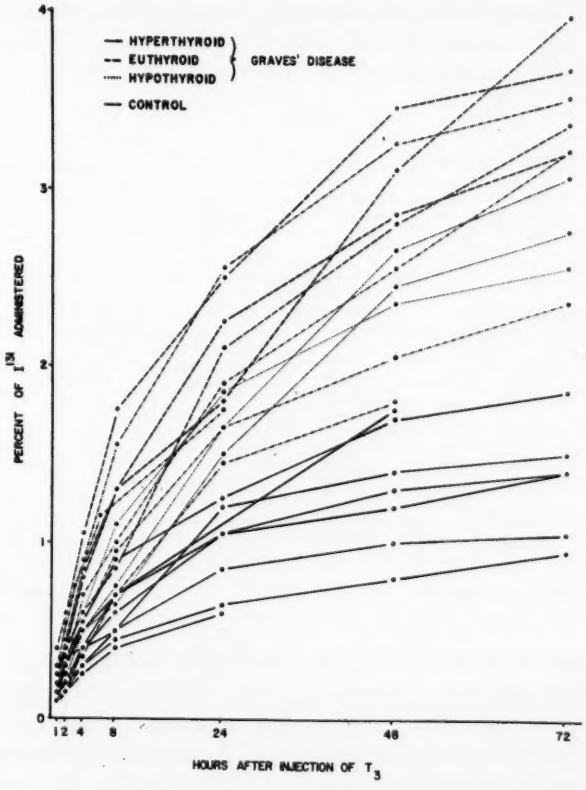

FIG. 2. Cumulative excretion of unaltered I^{131} triiodothyronine in the urine. More unaltered triiodothyronine was excreted by patients with Graves' disease than by the normal controls. The cumulative excretion in the two groups was different at twenty-four hours.

TABLE 1. SERUM LEVELS OF TRIIODOTHYRONINE IN GRAVES' DISEASE VERSUS CONTROLS

The level in patients with Graves' disease was higher at one hour than in controls ($P < .05$). The absence of values in the 48-hour and 72-hour columns is due to the fact that the radioactivity in the serum was too low for satisfactory chromatography.

Group	Case No.	Serum levels of triiodothyronine (% dose/liter						
		1 hour	2 hours	4 hours	8 hours	24 hours	48 hours	72 hours
Graves' Disease								
Hyperthyroid	9	9.0	8.0	6.8	2.4	0.75	0.32	0.20
	11	4.9	3.4	3.2	2.1	1.0	0.39	—
	18	13.2	9.2	6.6	3.5	0.8	0.4	—
	22	5.3	3.5	2.3	1.25	0.35	0.14	—
	Mean	*8.1±3.9*	*6.0±3.0*	*4.7±2.1*	*2.3±1.0*	*0.72±0.77*	*0.31±0.12*	—
Euthyroid	2	22.2	—	14.0*	7.9**	1.7	—	—
	6	5.5	5.3	4.1	2.5	1.5	0.3	0.18
	17	9.4	7.9	5.4	3.1	1.6	0.7	—
	19	7.2	5.6	3.3	1.7	0.5	—	—
	20	8.5	6.4	4.2	3.0	0.8	0.2	—
	Mean	*10.6±6.7*	*6.3±1.2*	*4.2±0.9*	*2.6±0.6*	*1.2 ±0.54*	*0.4 ±0.26*	—
Hypothyroid	13	9.9	8.0	4.7	3.6	1.0	—	—
	21	13.3	10.0	7.4	3.6	1.3	0.55	0.50
	16	11.0	6.5	7.2	4.7	2.7	1.4	—
	Mean	*11.4±1.7*	*8.2±1.7*	*6.4±1.5*	*4.0±0.6*	*1.7 ±0.9*	*0.98±0.60*	—
Total, Graves' disease	*Mean*	*10.0±4.7*	*6.7±2.1*	*5.0±1.8*	*2.5±1.0*	*1.2 ±0.6*	*0.49±0.38*	—
Controls								
	5	4.6	—	2.2	1.04	0.56	—	0.50
	12	6.8	4.4	3.1	1.4	1.0	0.6	0.2
	14	6.8	5.0	3.0	1.4	0.3	0.2	0.1
	15	11.6	8.0	4.3	2.8	—	—	—
	24	3.8	2.7	1.45	0.9	0.27	0.08	—
	25	4.5	3.1	1.7	1.0	0.35	—	—
	26	3.4	2.3	1.7	0.68	0.1	—	—
	27	4.9	3.5	2.4	1.18	0.52	0.23	—
	Mean	*5.8±2.6*	*4.1±1.9*	*2.5±1.0*	*1.3±0.6*	*0.44±0.29*	*0.28±0.22*	—

* Three hours
** Six hours
} not used in calculations.

Graves' disease were not only higher, but there was a delay in the fall of the curve. The difference between patients with Graves' disease and the controls became less impressive beyond eight hours, and by forty-eight hours both groups retained an average of 5 per cent (5.3 and 5.0) of the first-hour level of triiodothyronine.

The only radioiodinated compounds found in more than 100 chromatograms of butanol extracts of the serum from both patients with Graves' disease and controls, were triiodothyronine and iodide. In the system of

chromatography used, the presence of trace quantities of the iodinated acetic and pyruvic acid derivatives of thyronine could be completely excluded owing to the presence of relatively large amounts of triiodothyronine in the chromatograms.

There was an immediate rise in radioactivity over the liver after the injection of triiodothyronine. Indeed, it was so rapid that a maximal level was reached within three minutes following the injection. A decline thereafter was detectable in thirty minutes.

In 5 patients with Graves' disease (3 hyperthyroid, 1 euthyroid, and 1 hypothyroid) the radioactivity of the liver was estimated by the continuous recording method; in all, there was a secondary rise in radioactivity between four and seven hours after the injection of the triiodothyronine (Fig. 3). This confirmed similar findings demonstrated by more crude methods used in the first 7 patients with Graves' disease studied. Six controls were studied by the improved method, and in none was a secondary rise observed.

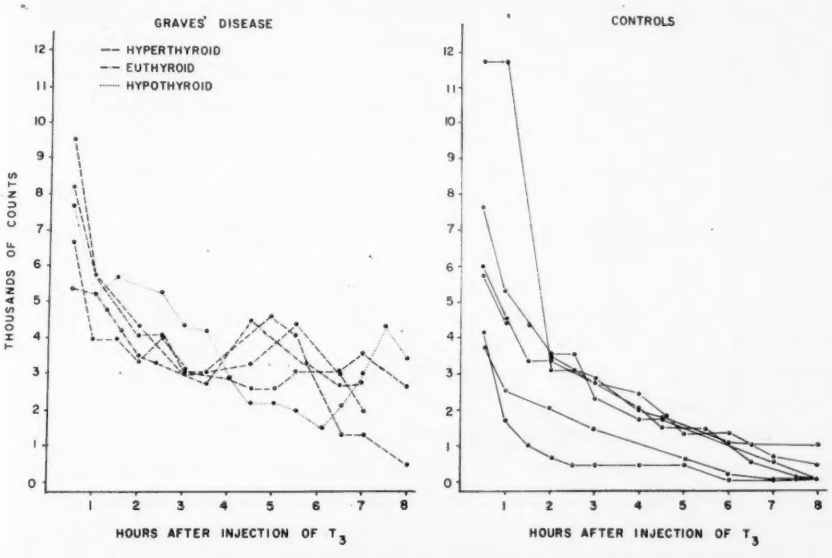

FIG. 3. Radioactivity in the region of the liver following injection of triiodothyronine. Radioactivity over the liver was corrected on the basis of a common dose. Maximal radioactivity was observed over the liver in approximately three minutes, and there was considerable reduction in the first hour. Data on the 11 patients studied with continuous recording and improved geometry are shown. The curves representing radioactivity in the livers of patients with Graves' disease showed a secondary rise in radioactivity four to seven hours after radioactive triiodothyronine was injected. A similar rise was also demonstrated in earlier patients studied by a more crude method.

Although the radioactivity in the liver began to fall soon after injection of the labeled triiodothyronine, the amount in the liver compared to the radioactivity in the serum was gradually rising. Since the radioactivity remained higher in the serum of patients with Graves' disease than in controls, it was interesting that the radioactivity of the liver in the two groups was not correspondingly different. Thus, the livers of patients with Graves' disease took up (or held) less of the available radioactivity than did the livers of the controls. To demonstrate this relationship the following ratio was devised:

$$\frac{\text{Counts per minute over liver (corrected for the dose)}}{\text{Triiodothyronine in serum (\% dose per liter)}}$$

The ratio at one hour in patients with Graves' disease was 3.9 ± 1.6, and in controls 4.8 ± 0.7. For purposes of illustration it seemed appropriate to

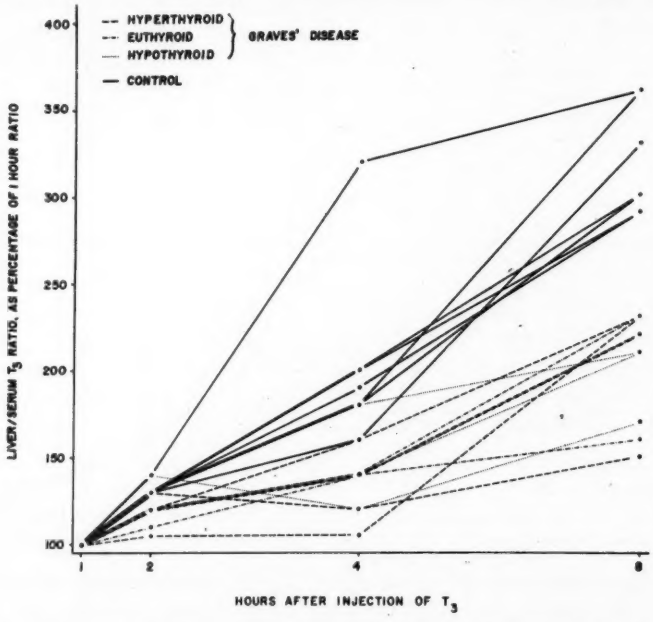

FIG. 4. The ratio of the radioactivity of the liver to the amount of triiodothyronine in the serum. The ratio is calculated as count over liver/amount of triiodothyronine in serum, expressed as a percentage of the dose per liter. This ratio is expressed as 100 per cent at one hour; at subsequent times it is expressed as a percentage of this one-hour ratio. The ratio was lower in patients with Graves' disease. At eight hours the mean values were: Graves' disease, 200 ± 31 per cent; controls, 320 ± 31 per cent. The difference is significant ($P < .005$). Thus, the radioactivity in the liver was less than might be expected in Graves' disease, considering the amount of circulating triiodothyronine.

express the changes in this ratio as deviations from the one-hour value, as shown in Figure 4.

The ratios in 17 subjects[5] were computed for 1, 2, 4 and 8 hours after injection of triiodothyronine. Figure 4 serves to illustrate a difference in the liver/serum-triiodothyronine ratios between the two groups of patients. Thus, although the serum contained more triiodothyronine in the patients with Graves' disease than in the controls, the liver either did not collect, or destroyed and released more readily, the radioactivity that came to it. The fact that the urinary excretion data showed increased amounts of free iodide may indicate the latter was taking place.

Counting over the thyroid area revealed no significant accumulation of radioactivity. Furthermore, the failure to find labeled thyroxine in the chromatograms of the serum of any subject indicates that the thyroid did not secrete appreciable amounts of iodinated organic compounds.

DISCUSSION

The patients of this study were known to have had Graves' disease. At the time the studies were performed, 4 of them were still hyperthyroid (duration, twelve to thirty-four months), 5 were euthyroid (duration of remission, six months to two years), and 2 were hypothyroid (duration, six to eighteen months). We do not know the underlying cause of Graves' disease, and although we can satisfactorily treat the hyperthyroidism, some of the facets of this clinical entity remain for some time after the patient becomes euthyroid. In spite of the widely different metabolic states within this group of patients, the results were essentially similar in all, but quite different from the results in the normal controls.

The only apparent significant difference in the values among the subgroups of Graves' disease was with regard to the amount of unaltered triiodothyronine excreted in the urine. More triiodothyronine was excreted by the hyperthyroid than by the hypothyroid patients. Renal plasma flow, inulin clearance, glomerular filtration, and para-aminohippuric acid excretion are all known to be elevated in hyperthyroidism and decreased in hypothyroidism (20). Thus, the slight difference in the amounts of triiodothyronine excreted by the kidney in these groups may have been attributable to the general metabolic state as reflected in renal function and the clearance of triiodothyronine. It should be emphasized that these differences in the amounts of unaltered excreted triiodothyronine within the Graves' disease group, although relatable to the degree of metabolism, are far less significant than the striking difference in values between the

[5] During the course of this study, the method of measuring the radioactivity of the liver was revised several times. Data on the last 17 subjects studied were sufficiently complete to permit inclusion in this analysis.

Graves' disease patients as a group and the normal subjects who were all in a euthyroid state. Thus, even when thyroid function had recently been so seriously impaired in Graves' disease that myxedema had developed, more unaltered triiodothyronine was still excreted than in the normal controls who were in a euthyroid state.

At the end of the first hour and until eight hours, the level of triiodothyronine remained higher in the serum of patients with Graves' disease than in the controls. These higher levels in the serum suggest that the rate of destruction of triiodothyronine, or its sequestration by the tissues, was delayed in the Graves' disease group. Seemingly conflicting observations are the fact that the different serum levels of triiodothyronine approached the same values after eight hours, and that considerably larger amounts of free labeled iodide were excreted in the patients with Graves' disease. The only available explanation for these conflicting findings is that the triiodothyronine which was removed from the circulation in patients with Graves' disease was then more rapidly deiodinated than in the normal subjects. The nature of the binding of triiodothyronine and other iodinated compounds by serum proteins may have some bearing on the disappearance of triiodothyronine from the serum, clearance by the kidney, and disposition in the tissues. These possibilities were considered but not explored specifically in our patients.

Roche *et al.* (14, 15) and Flock *et al.* (16–18) showed in animals that the liver plays a role in the metabolism of triiodothyronine. Ruegamer and Chodos (21) found that the half-life of triiodothyronine in the serum is prolonged in humans with liver disease. The same was shown with regard to thyroxine by Vannotti and Béraud (22). In our study, the amount of triiodothyronine in the serum did not fall as rapidly in the patients with Graves' disease as it did in the normal controls. Even though the serum triiodothyronine levels were higher, the amount of radioactivity in the liver was relatively lower than in the controls, as shown by the liver/serum-triiodothyronine ratios (Fig. 4). Although the actual amount of radioactivity attributable to the liver was small and the secondary rise observed in the liver was of short duration, the consistency of this secondary rise in all patients with Graves' disease makes the finding noteworthy. This, together with the delay in the decline of the serum triiodothyronine level and the lower liver/serum-triiodothyronine ratios, suggests that an alteration in the function of the liver may be a feature of Graves' disease.

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REFERENCES

1. Gross, J., and Pitt-Rivers, R.: The identification of 3:5:3′-L-triiodothyronine in human plasma, *Lancet* **1**: 439–441 (March 1) 1952.
2. Gross, J., and Pitt-Rivers, R.: Physiological activity of 3:5:3′-L-triiodothyronine, *Lancet* **1**: 593–594 (March 22) 1952.
3. Gross, J.; Pitt-Rivers, R., and Trotter, W. R.: Effect of 3:5:3′-L-triiodothyronine in myxoedema, *Lancet* **1**: 1044–1045 (May 24) 1952.
4. Rall, J. E.; Robbins, J.; Becker, D., and Rawson, R. W.: The metabolism of labeled *l*-triiodothyronine, *l*-thyroxine, and *d*-thyroxine, (abstract) *J. Clin. Invest.* **32**: 596 (June) 1953.
5. Sterling, K.; Lashof, J., and Man, E. B.: The turnover rate of *l*-thyroxine and *l*-triiodothyronine in euthyroid subjects as measured by radioiodine-labeled compounds, *Clin. Res. Proc.* **2**: 41 (March) 1954.
6. Michel, R., and Pitt-Rivers, R.: The relative potencies of thyroxine and triiodothyronine analogues *in vivo*, *Biochim. et biophys. acta* **24**: 213–214 (Apr.) 1957.
7. Maclagan, N. F.; Bowden, C. H., and Wilkinson, J. H.: The metabolism of thyroid hormones. 2. Detection of thyroxine and tri-iodothyronine in human plasma, *Biochem. J.* **67**: 5–11 (Sept.) 1957.
8. Benua, R. S.; Dobyns, B. M., and Ninmer, A.: Triiodothyronine in the serum of patients treated with radioactive iodine, *J. Clin. Endocrinol. & Metab.* **15**: 1367–1378 (Nov.) 1955.
9. Benua, R. S., and Dobyns, B. M.: Iodinated compounds in the serum, disappearance of radioactive iodine from the thyroid, and clinical response in patients treated with radioactive iodine, *J. Clin. Endocrinol. & Metab.* **15**: 118–130 (Jan.) 1955.
10. Lerman, J.: The physiologic activity of *l*-triiodothyronine, *J. Clin. Endocrinol. & Metab.* **13**: 1341–1346 (Nov.) 1953.
11. Pitt-Rivers, R., Stanbury, J. B., and Rapp, B.: Conversion of thyroxine to 3-5-3′-triiodothyronine *in vivo*, *J. Clin. Endocrinol. & Metab.* **15**: 616–620 (May) 1955.
12. Lassiter, W. E., and Stanbury, J. B.: The *in vivo* conversion of thyroxine to 3:5:3′-triiodothyronine, *J. Clin. Endocrinol. & Metab.* **18**: 903–906 (Aug.) 1958.
13. VanArsdel, P., Jr.; Hogness, J. R.; Williams, R. H., and Elgee, N.: Comparative distribution and fate of I^{131}-labeled thyroxine and triiodothyronine, *Endocrinology* **55**: 332–343 (Sept.) 1954.
14. Roche, J.; Michel, R., and Tata, J.: Sur l'excretion biliaire de la glycuroconjugaison de la 3:5:3′-triiodothyronine, (The biliary excretion of the glucuronic conjugate of 3,5,3′-triiodothyronine), *Compt. rend. Acad. d. Sc.* **236**: 1614–1616 (Apr. 20) 1953.
15. Roche, J.; Michel, O.; Michel, R., and Tata, J.: On the products of hepatic and renal elimination of thyroxine ant triiodothyronine labelled with iodine-^{131}, *in* Radioisotope Conference 1954: Vol. I, Medical and Physiological Applications, ed. by J. Johnston, R. Faires and R. Millett. London, Butterworth & Co., Ltd., 1954, pp. 325–329.
16. Flock, E. V.; Bollman, J. L., and Grindlay, J. H.: Biliary excretion and metabolism of radioactive L-triiodothyronine, *Am. J. Physiol.* **189**: 420–424 (June) 1957.
17. Flock, E. V., and Bollman, J. L.: The metabolism of thyroxine and triiodothyronine in the eviscerated rat, *J. Biol. Chem.* **214**: 709–721 (June) 1955.
18. Flock, E. V.; Bollman, J. L.; Grindlay, J. H., and Orvis, A. L.: Metabolism of

L-thyroxine and L-triiodothyronine in the absence of the liver, *Am. J. Physiol.* **187:** 407–414 (Dec.) 1956.

19. HALES, I. B.: A method for the identification and measurement of the iodinated organic compounds of urine. Submitted for publication to Journal of Endocrinology (London).
20. HLAD, C. J., JR., and BRICKER, N. S.: Renal function and I^{131} clearance in hyperthyroidism and myxedema, *J. Clin. Endocrinol. & Metab.* **14:** 1539–1550 (Dec.) 1954.
21. RUEGAMER, W. R., and CHODOS, R. B.: Diiodotyrosine and triiodothyronine metabolism in liver disease, *Clin. Res. Proc.* **4:** 145 (Apr.) 1956.
22. VANNOTTI, A. and BÉRAUD, T.: Functional relationships between the liver, the thyroxine-binding protein of serum, and the thyroid, *J. Clin. Endocrinol. & Metab.* **19:** 466–477 (Apr.) 1959.

A SENSITIVE METHOD FOR THE DETECTION OF IODINATED COMPOUNDS IN HUMAN PLASMA, AND ITS APPLICATIONS IN THE STUDY OF THYROTOXICOSIS*

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ABSTRACT

Iodinated tyrosines were identified in human plasma in certain familial disorders, after the large doses of radiation given in some cases of carcinoma of the thyroid and, as previously reported by this department, in some, but not all cases of thyrotoxicosis. The evidence for this statement is further strengthened by the results obtained with a modification of a chemical method for the detection of small amounts of iodinated compounds which were separated by paper chromatography. This method depends essentially on the treatment of paper chromatograms with ceric sulphate-arsenious acid reagent and is a development of that described by Bowden *et al.* (*Biochem. J.* **59**: 93, 1955). Using this technique alone, and in conjunction with scintillation counting, it was possible to study the plasma of patients before and after the administration of radioiodine. In this way a radiation effect on the thyroid may be excluded as the cause for the appearance of abnormal iodinated compounds in the plasma of certain patients with thyrotoxicosis. Iodinated compounds in the plasma of normal people were examined.

INTRODUCTION

THE use of radioiodine, combined with the development of chromatographic techniques, has permitted extensive study of the nature of the circulating thyroid hormone (1, 2). However, the detection of iodinated compounds in plasma after the administration of radioiodine raises the possibility that they may, at least in part, be radiation artefacts. Various workers (3, 4) have developed methods of chromatography using minimal amounts of radioactivity to reduce this possibility.

The introduction of the highly sensitive ceric sulphate-arsenious acid reaction for the detection of iodinated compounds on paper chromatograms by Bowden *et al.* (5) enabled Maclagan and his co-workers (6) to apply this method to investigation of the circulating thyroid hormones, without using a radioactive technique.

Iodinated tyrosines have been detected in certain patients with thyro-

* Presented at the Annual Meeting of the American Goiter Association, Chicago, Illinois, April 30–May 2, 1959.

toxicosis after the administration of tracer amounts of 100–200 μc. of radioactive iodine (7) as well as after therapeutic doses in the same patients. It was found that the presence or absence of iodinated tyrosines in plasma was statistically related to the size of the thyroid gland. Thyrotoxic patients in whose plasma iodinated tyrosines were detected appear to require larger amounts of radioactive iodine to control the disease.

It seemed desirable to extend this investigation and to carry out parallel studies using both radioactive and non-radioactive methods. The chemical procedure used in this investigation is a modification of that described by Bowden *et al.* (5) and depends essentially on the treatment of paper chromatograms with ceric sulphate-arsenious acid reagent. To increase the sensitivity of the method, and to prevent fading of the background, the chromatogram is given a second treatment with a solution of aniline in acetone. Using this technique alone, and in conjunction with scintillation counting, it has been possible to study the plasma of patients before and after the administration of radioactive iodine. In this way the radiation effect on the thyroid may be excluded as a cause of the presence of abnormal iodinated compounds in the plasma. The iodinated compounds in the plasma of normal people who have not been given radioactive iodine were also examined.

MATERIALS AND METHODS

Materials and patients

For this investigation chromatograms were prepared from the butanol-extractable fraction of plasma samples. Blood was drawn into heparinized bottles. The blood was centrifuged at 2,500 rpm for ten minutes and the plasma taken off; when necessary, it was kept in the refrigerator until needed. Blood was taken from 19 normal controls without any suspected thyroid disorder, who were having routine medical examination for other reasons. Blood was also taken from all patients suffering from thyrotoxicosis who were having, or were about to have, treatment with radioactive iodine. From a smaller number of previously untreated thyrotoxic patients, blood samples were obtained before any treatment was prescribed.

Methods

An aliquot of plasma measuring 4–6 ml. was washed three times rapidly with equal volumes of carbon tetrachloride, and the washings rejected. The washed plasma was extracted with n-butyl alcohol and the extract evaporated to dryness and taken up in a mixture of butanol and water as previously described (3). The extract was applied to 10-inch × 10-inch squares of 3 MM Whatman filter paper and run overnight in butanol: ethanol: 2*N* ammonia (5:1:2) solvent. After drying, the product was run again at right angles, overnight, in a second solvent system of butanol: acetic acid: water (12:3:5): The dried chromatograms were then treated chemically. The solutions required were.

1. Two per cent ceric sulphate in *N* H_2SO.
2. Arsenious acid, 0.2 *N*. Arsenious acid is made up as follows: 9.891 Gm. of arsenious trioxide and 7 Gm. of sodium hydroxide are dissolved in 100 ml. of water and then

diluted to 400 ml. with more water. Concentrated H_2SO_4 is added until the solution is about pH 7 when judged by means of indicator paper. A further 42 ml. of concentrated H_2SO_4 is added before diluting the solution to 1 liter with water. The final acidity is 1.5 *N*.

3. Ceric sulphate-arsenious acid solution. This solution is prepared immediately before treating the chomatogram. The proportions are solutions 1, 2 and water (1:1:1 v/v)
4. One per cent aniline in acetone.

The chromatograms were dipped quickly in solution 3, gently blotted and hung up to dry at room temperature in a fume cupboard. The air was kept circulating during this process. When the chromatograms had dried they were dipped in solution 4 and hung again in the fume cupboard to dry at room temperature. Radioactive chromatograms were cut into strips 3 cm. wide and counted between two scintillation counters (3). If the initial activity on the chromatogram was more than 0.2 μc. of I^{131} they were also put on to x-ray films.

RESULTS

The sensitivity of the method is such that on chromatograms of standard solutions, amounts of the order of 0.04 μg. of *l*-thyroxine, triiodothyronine, diiodotyrosine and monoiodotyrosine can be detected. It is usually accepted that plasma from healthy people contains 4–8 μg. of protein-bound iodine (PBI^{127}) per 100 ml., whereas plasma from patients suffering from thyrotoxicosis contains up to 20 μg. or more of PBI^{127} per 100 ml.

The method of radioactive chromatography discussed here is not sufficiently sensitive to enable any analysis to be made of plasma from normal controls. It would be necessary to administer large amounts of radioactivity in order to identify I^{131}-labelled compounds on chromatograms, and the possibility of radiation effects could not then be ignored. For thyrotoxic patients, however, in whom the level of PBI^{131} is from 0.5 to 4 per cent of the administered radioactivity per liter of plasma, it is possible to determine the relative proportions of the different iodinated compounds found. On the whole, our results agree well with those reported by other workers (8) and are of the order of:

Not extractable by n-butanol	10–20%
Thyroxine	40–70%
Triiodothyronine (when identified)	up to 30%
Free iodide	less than 20%
Iodinated tyrosines (when found)	about 5%

If only about 5 per cent of the PBI^{127} is in the form of iodinated tyrosines, then in an extract of 6 ml. of plasma from a thyrotoxic patient there would be .03–.06 μg. of iodinated tyrosines. From this it can be seen that the iodinated tyrosines, even if present, will be detected with this method in only a small proportion of cases. In a few patients with high levels of PBI^{127} in the plasma, spots corresponding to diiodotyrosine or even mono-

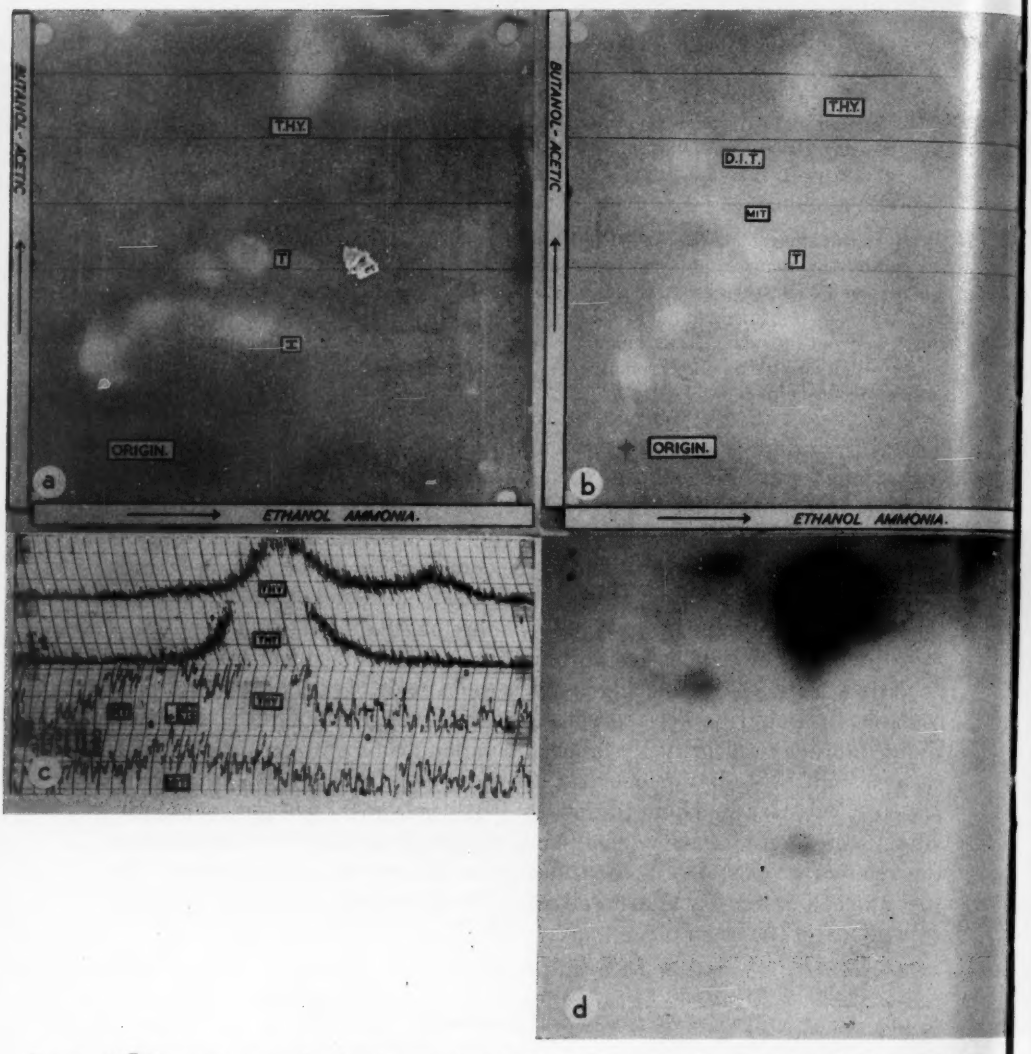

FIG. 1. (a) Chromatogram of extract equivalent to 6 ml. of plasma drawn forty-eight hours after the ministration of 22 mc. of I^{131}. (b) Chromatogram of 6 ml. of the *same* sample of plasma to which 1.0 μg. of diio tyrosine and 1.0 μg. of monoiodotyrosine have been added. (c) Counting record of (b). (d) Film of (b). T.H.Y. = Thyroxine. D.I.T. = Diiodotyrosine. M.I.T. = Monoiodotyros T = Tryptophan. I = Iodide.

iodotyrosine may be noted before any radioactive substances have been given. In other cases, iodinated tyrosines can be demonstrated by counting after the administration of 200 μc. of I^{131} when they cannot be demon-

strated by chemical means. Figure 1*a* shows the chromatogram of an extract of 6 ml. of plasma from a patient who had been treated with 22 mc. of I^{131} forty-eight hours previously. Figure 1*b* shows the chromatogram from the same plasma sample to which 1.0 μg. of diiodotyrosine and 1.0 μg. of monoiodotyrosine had been added.

In the extracts of plasma from normal control subjects, thyroxine, triiodothyronine, iodide, and sometimes a small amount of an unidentified but apparently iodinated compound were detected (Fig. 2).

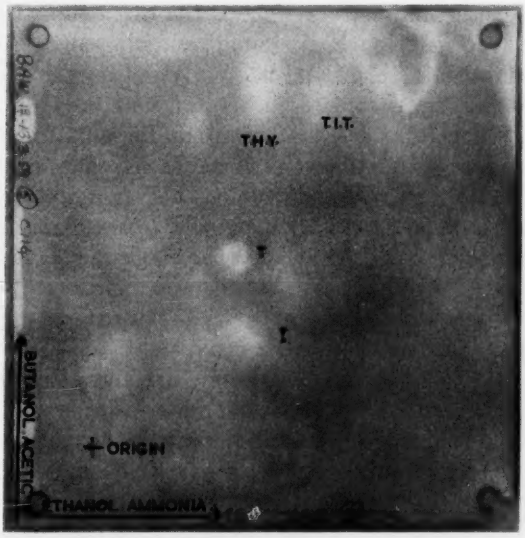

FIG. 2. Chromatogram of 4 ml. of normal plasma.

This iodinated compound was detected by chemical means and does not appear to be thyroxine, triiodothyronine, or mono- or diiodotyrosine. It was found on several chromatograms from both thyrotoxic and normal people. Chromatograms from radioactive plasma confirmed that the substance can contain radioactive iodine.

DISCUSSION

The combined application of chemical and radioactive techniques for the detection of iodinated compounds on paper chromatograms has certain advantages in studying the metabolism of thyroid hormone. However, before chemical detection could be applied routinely it was thought necessary to devise a method that was sufficiently sensitive, and not too time-consuming.

A disadvantage of treating chromatograms with ceric sulphate-arsenious

acid reagent for the detection of iodinated compounds is the fading of the background. After a few days the chromatogram may become completely colorless, and even while drying the yellow background tends to fade. In some chromatograms it becomes very pale and the definition of iodine spots poor. The introduction of a second treatment of the chromatogram with a solution of aniline in acetone greatly increases the contrast between background and iodine spots. It also facilitates the detection of iodinated compounds present in very small amounts, and those producing a weak reaction with ceric arsenious-acid solution. Under these conditions the reaction between the aniline solution and the ceric sulphate on the chromatogram produces a green background, and the contrast between the white iodine spots and background is greatly improved. The background does not fade, and the chromatograms may be stored for two to three months. A pink spot appears on the chromatograms immediately after treatment with ceric sulphate-arsenious acid reagent, and fades to a pale pink on standing. Added tryptophane causes a similar color reaction and migrates to the same position in the solvent systems used. The lipid content of the butanol extracts influenced the subsequent chromatography. Areas where lipid-containing material was present were pale brown. When a chromatogram was stained for lipids, in a solution of Sudan Black, the light brown areas took up the dye strongly.

Maclagan *et al.* (6) subjected the butanol extracts of plasma to a series of processes for the removal of fats and water-soluble substances. Their technique was too laborious to be applied in the investigation of large numbers of patients, and so the system of extraction and chromatography previously used in this hospital (3) was retained in the present investigation. The extracts applied to the paper consequently still contained appreciable amounts of lipid. The amount of lipid present varied with the plasma extracts. Satisfactory chromatographic separation was not always obtained. In good chromatograms the lipid-containing material ran as a distinct zone ahead of the thyroxine and triiodothyronine spots, and did not interfere with the separation of these compounds (Fig. 2). When the extracts had a high lipid content, streaking sometimes occurred and prevented accurate assessment of the chromatogram. In order to detect the small amounts of tyrosines both by chemical and radioactive methods, it was necessary to run extracts equivalent to 4–6 ml. of plasma. For the detection of thyroxine and triiodothyronine, extracts equivalent to 3–4 ml. of plasma were satisfactory, and the likelihood of lipid streaking was reduced. The sensitivity of the method approached that attained by Stolc's (9) modification of Bowden's method (5) for the detection of thyroxine.

Apart from its use for the detection of iodinated compounds in non-radioactive plasma, the pretreatment of radioactive chromatograms by

chemical means is of value when the chromatogram is to be counted for radioactivity. The iodinated compounds are detectable without the addition of markers, and provide a useful cross-check with the radioactive scan.

We have found, in agreement with other workers, that thyroxine and triiodothyronine are the main iodinated compounds in plasma. Under the conditions of this investigation, iodinated tyrosines were not usually detected on the chromatograms. Iodinated tyrosines were, however, found both by radioactive and chemical methods in the plasma in certain cases of thyrotoxicosis. In a few thyrotoxic patients they were detected by chemical methods before any radioactive iodine was given. Their presence was subsequently confirmed by both radioactive and nonradioactive methods after a tracer of 200 μc. of I^{131} and again following treatment with I^{131}. The fact that they were detected before the administration of any radioactivity supports the hypothesis that they were not produced only as a result of irradiation of the thyroid. That they were produced as artefacts during the process of butanol extraction or subsequent chromatography cannot be excluded. However, if this be so, it would seem that they are produced only in the plasma of certain thyrotoxic patients, and that they consistently appear in serial samples from the same patients. When pure thyroxine and triiodothyronine were added to normal plasma before processing, no iodinated tyrosines could be seen on the chromatogram, although the added substances could easily be detected.

Farran *et al.* (7), using techniques dependent upon the administration of radioiodine, found iodotyrosines in the plasma of certain patients suffering from thyrotoxicosis. They also found that these patients required larger amounts of radioiodine to control the disease.

The method described, used alone and in combination with techniques dependent upon the use of radioiodine, provided useful qualitative information concerning the nature of iodinated compounds in butanol extracts of plasma.

Although iodinated tyrosines were detected in the extracts of plasma from only a few cases of thyrotoxicosis, and in very small amounts, their presence or absence may be of considerable importance in the study of the etiology of thyrotoxicosis.

REFERENCES

1. Taurog, A.; Chaikoff, I. L., and Tong, W.: The nature of plasma iodine as revealed by filter paper partition chromatography, *J. Biol. Chem.* **184:** 99, 1950.
2. Gross, J., and Pitt-Rivers, R.: The identification of 3:5:3′:L-triiodothyronine in human plasma, *Lancet* **1:** 439, 1952.
3. Farran, H. E. A.; Parker, V., and Abbatt, J. D.: Low activity chromatography in radioactive iodine studies, *Brit. J. Radiol.* **30:** 445, 1957.

4. Block, R. J.; Werner, S. C., and Mandl, R. H.: A method for the investigation of the distribution of radioiodine in the serum after small test doses of I^{131}, *Arch. Biochem. & Biophys.* **73**: 9, 1958.
5. Bowden, C. H.; Maclagan, N. F., and Wilkinson, J. H.: The application of ceric sulphate-arsenious acid reaction to the detection of thyroxine and related substances, *Biochem. J.* **59**: 93, 1955.
6. Maclagan, N. F.; Bowden, C. H., and Wilkinson, J. H.: The metabolism of thyroid hormones, *Biochem. J.* **67**: 5, 1957.
7. Farran, H. E. A.; Lea, A. J.; Goolden, A. W. G., and Abbatt, J. D.: The presence of iodinated tyrosines in the plasma of patients with thyrotoxicosis, *Lancet* **1**: 793, 1959.
8. Werner, S. C., and Block, R. J.: Discrepancy between the distributions of iodine in human serum when estimated by $iodine^{131}$ and $iodine^{127}$, *Nature* **183**: 406, 1959.
9. Stolc, V.: Chromatographic detection of minute quantities of thyroxine in serum, *Nature* **182**: 52, 1958.

OCCULT PAPILLARY CARCINOMA OF THE THYROID GLAND: A STUDY OF 140 CASES OBSERVED IN A 30-YEAR PERIOD*

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ABSTRACT

Of 140 occult papillary carcinomas treated surgically at the Mayo Clinic over a 30-year period, 58 were associated with nodal metastasis and 82 were found incidental to thyroid operations for other conditions. Of the 58 patients with nodal metastasis, 4 have died from causes unrelated to thyroid carcinoma and 1 patient is untraced. The remainder are alive, without evidence of carcinoma, three to thirty-two years after operation. Of the 82 patients without metastasis, 11 are dead of known causes other than thyroid carcinoma. The remainder are living after periods ranging up to thirty years, and in none is either local or distant metastasis known to have developed, although a few differences were noted. Histologically, no clear-cut line could be drawn between those lesions associated with nodal metastasis and those discovered incidental to operation for a variety of thyroid disorders.

THE occurrence of a minute, clinically occult, papillary carcinoma of the thyroid gland in association with large cervical nodal metastasis is today a well-recognized pathologic entity. Prior to the last decade or so, however, much confusion existed in regard to this subject, and in the absence of a readily detectable primary lesion the concept of "lateral aberrant thyroid tumor" was widely accepted as an explanation for the nodal metastasis in the neck (1). The correct explanation of this phenomenon (2, 3) focused attention on small papillary tumors of the thyroid gland in general, both those found in association with nodal metastasis and those discovered incidental to surgical treatment for a variety of pathologic conditions of the thyroid. Study of a group of the latter type with follow-up data indicated a benign behavior, and the name "nonencapsulated sclerosing tumor" was suggested (4). In a second study, selection of 32 apparently

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similar cases included 10 with metastasis to regional nodes and the term "occult sclerosing carcinoma" was advanced (5).

Careful study of thyroid glands removed at necropsy has demonstrated a surprising incidence of minute papillary lesions which may be identical in structure with the tiny primary carcinomas found associated with cervical nodal metastasis (6). In addition, more careful examination and sectioning of small lesions in thyroid glands removed at operation have, in recent years, provided us with a considerably more frequent diagnosis of "occult papillary carcinoma" of the thyroid.

A recent survey of the results of our treatment of papillary carcinoma over a 10-year period (7) indicated an exceptionally favorable outlook for patients with these small tumors. In the light of the relative scarcity of long-term follow-up data on these slowly growing lesions, all small papillary carcinomas found in surgically treated patients over a 30-year period were reviewed. The object of the study was twofold: 1) to compare the morphologic features of a group of occult primary papillary carcinomas detected by means of nodal metastasis with a somewhat larger group detected incidentally at thyroid operations of various types, and 2) to obtain long-term follow-up data on both groups and to ascertain whether or not rather conservative surgical management has provided satisfactory treatment.

MATERIAL AND METHOD

The selection of cases for the study of "occult papillary carcinoma" involved a careful review of all papillary carcinomas of the thyroid gland treated surgically at the Mayo Clinic over the period 1926 through 1955. All gross specimens were re-examined and additional microscopic sections were prepared as necessary. Special attention was given to size of the primary tumor, character of the thyroid parenchyma, and size, number and appearance of metastatically involved lymph nodes. Most small clinically nonpalpable papillary carcinomas discovered on the basis of large metastatically involved cervical lymph nodes were 3 mm. to 1 cm. in diameter. However, in a few cases the primary tumor was somewhat larger and an arbitrary upper limit of size of 1.5 cm. in greatest diameter was selected. Carcinomas that exceeded this dimension were excluded. In a similar fashion, all small papillary carcinomas found by the surgical pathologist in thyroid glands resected primarily for nodular goiter, Graves' disease, thyroiditis and so forth were included provided the lesion did not exceed 1.5 cm. in diameter.

Virtually all such carcinomas in both categories were not palpable on clinical examination. In a few cases the primary lesion was clinically detected or suspected by reason of its superficial position in the isthmus or pyramidal lobe or near the surface of the thyroid gland. These cases were included since they fell within the range of size selected as ordinary "occult" carcinoma. Also included were 6 cases in which large metastatic nodal deposits were removed surgically from the neck but in which no thyroid tissue was resected. In 4 of these the thyroid lobe on the involved side was actually inspected and palpated at the time of operation and called normal. In the other 2 no tumor was felt in the thyroid on clinical examination. The majority of these cases were seen in

the earlier decades of the study when the concept of "lateral aberrant thyroid" as an explanation of the phenomenon of nodal metastasis was widely accepted.

INCIDENCE

On the basis of the foregoing criteria, 140 cases of small or occult papillary carcinoma of the thyroid gland were discovered in the 30-year period. Of these patients, 58 (41 per cent) had associated metastasis to the lymph nodes when first seen; 82 had no nodal metastasis and their lesions were, in the main, discovered incidentally at operations for a variety of pathologic conditions of the thyroid gland. During this same 30-year period, approximately 550 patients with papillary carcinoma were treated surgically at the Mayo Clinic.

AGE AND SEX

The average age in the entire series was 40.5 years with a range from 6 to 72 years. The average age of the 58 patients with nodal involvement was 34 years, and of those without nodal involvement, 45 years. The distribution of the patients by age is shown in Table 1. There were 94 females and 36 males. The ratio of females to males was 3:2 for the patients with metastasis to the nodes, and 6:1 for those without metastasis.

CLINICAL FINDINGS

In general, the presenting symptom of the patients with nodal metastasis was that of a lump in the lateral part of the neck. The length of time the mass had been present varied from two weeks to eleven years, with an average of two years and nine months. In only 2 cases was a goiter present

TABLE 1. AGE DISTRIBUTION OF PATIENTS WITH OCCULT PAPILLARY CARCINOMA OF THYROID

Age (yrs.)	Metastasis to nodes	
	Yes	No
0–9	4	0
10–19	6	3
20–29	15	8
30–39	16	10
40–49	8	26
50–59	4	30
60–69	4	4
70–79	1	1
Total	58	82

in addition to the cervical adenopathy. Clinical examination disclosed the goiter in these 2 cases and slight enlargement of the thyroid in an additional 6 cases. In only 2 cases was the small primary tumor definitely palpable. A preoperative histologic diagnosis of papillary carcinoma of the thyroid was made on the basis of a cervical node excised elsewhere in 22 cases. In the remainder, preoperative impressions included metastatic carcinoma of the thyroid, branchial-cleft cyst, malignant lymphoma, tuberculous adenitis, indeterminate lymphadenopathy and tumor of the neck.

The preoperative clinical diagnosis was nontoxic nodular goiter in 46 of the patients without nodal metastasis, toxic nodular goiter in 12, Graves' disease in 19, probably thyroiditis in 2, thyroglossal-duct cyst or cyst of the neck in 2 and hyperparathyroidism in 1.

PATHOLOGIC FINDINGS

Location and multiplicity of lesion. The thyroid gland was available for study in 134 of the 140 cases. The carcinoma was in the right lobe in 60 cases, in the left lobe in 45, in the isthmus or pyramidal lobe in 8, and bilateral in 3. In the other 18 cases, including 2 cases of multicentric lesions, the side involved was not specified and it was not possible to determine this from a study of the gross specimen. The lesions were single in 121 cases and multiple in 13 cases. In the latter group, 11 patients each had two separate small carcinomas, and 2 patients each had three. Data on the 6 cases in which thyroid tissue was not removed are given later.

Size of lesion. The range of size in this group of carcinomas is illustrated in Figure 1. The smallest lesion associated with nodal metastasis measured 3 mm. in diameter, and the largest, 1.4 cm. In the patients without nodal metastasis the smallest lesion measured 3 mm. in diameter, and the largest, 1.5 cm. The average diameter of the entire group of 149 small carcinomas was 0.76 cm. The average diameter was almost the same in the patients with, as in those without, nodal involvement. In the group with nodal involvement, 51 (88 per cent) were 1 cm. or less in diameter; in those without nodal involvement, 71 (87 per cent) were 1 cm. or less in diameter.

Parenchymal findings. In the patients with nodal involvement the thyroid parenchyma was typically normal on both gross and microscopic examination. Two thyroid glands showed the gross nodularity of nontoxic nodular goiter and 2 additional glands contained a single adenoma in addition to the small papillary carcinoma. Two showed an appreciable degree of lymphocytic thyroiditis and 1 presented the typical gross and microscopic features of Graves' disease. In 6 cases no parenchyma was available for study.

The major pathologic findings were those of nontoxic nodular goiter in 22 of the patients without nodal involvement, single adenoma in 12,

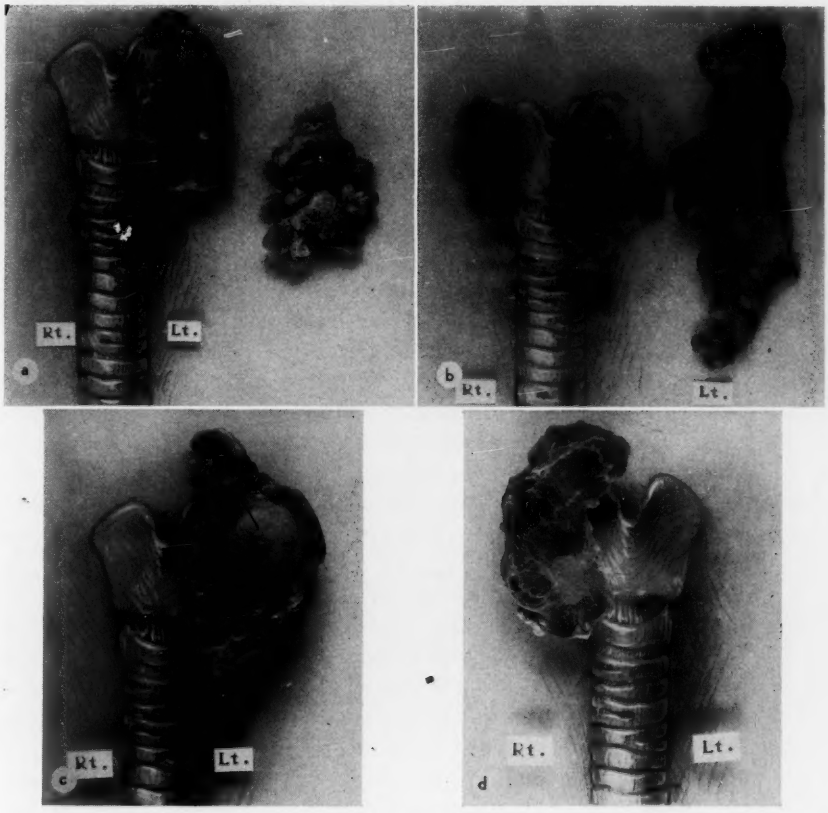

FIG. 1. Occult papillary carcinoma of thyroid. **a.** Very small (3 mm.) carcinoma with moderate-sized nodal metastatic lesion. **b.** Very large left jugular nodal metastatic lesion with primary lesion 1.4 cm. in greatest diameter. The thyroid lesion was not palpable on clinical examination. **c.** Small lesion (arrows) 0.8 cm. in diameter located in thyroid parenchyma adjacent to large fetal adenoma. **d.** Papillary carcinoma 1.5 cm. in diameter in a macrofollicular, slightly nodular parenchyma.

Graves' disease in 19, toxic nodular goiter in 9, lymphocytic thyroiditis in 9, and "colloid" goiter in 1. In these 72 cases the carcinoma was not palpable on clinical examination and was discovered by the surgical pathologist on examination of the surgical specimen. In a few cases this was accomplished, not at the time of operation but on review of the specimen in a subsequent study. In the remaining 10 cases the thyroid parenchyma was virtually normal and the small carcinoma was clinically palpable. In 5 of these the tumor was present in the isthmus or pyramidal lobe and in the other 5 it was in the superficial portion of the right or left lobe of the thyroid.

Nodal metastasis. Involvement of multiple regional nodes was noted in 52 of the 58 patients with nodal metastasis, and in the remaining 6 a single large node was metastatically involved. In many instances the enlargement of nodes was massive, producing a huge aggregate of metastatic tissue which contrasted in striking fashion with the minute primary carcinoma. The jugular chain was the site involved most frequently, followed by the tracheo-esophageal nodes and nodes in the vicinity of the thyroid capsule. Rarely, affected nodes were removed from the posterior triangle of the neck or upper part of the mediastinum. Bilateral affected nodes were removed in 3 cases. On examination the involved nodes measured up to 4 or 5 cm. in diameter, were frequently grossly cystic and papillary, and rarely contained foci of calcification. As a rule, the nodes could be readily removed from the surrounding tissue.

Microscopic findings. As is true of ordinary-sized papillary carcinomas of the thyroid gland, the microscopic features of these small tumors varied considerably. In general, all were unencapsulated tumors showing a predominantly papillary, predominantly follicular, or mixed architecture and exhibiting a varied degree of sclerosis with or without psammoma bodies (Fig. 2).

An attempt was made to discover any basic histologic differences between the group with and the group without nodal metastasis (Figs. 3 and 4). For this purpose, the lesions were compared and graded as to the degree of invasiveness of tumor into the adjacent parenchyma, predominant architecture, amount of sclerosis and the presence or absence of psammoma bodies. No appreciable difference in the degree of invasiveness was noted between the two groups. In 46 per cent of the patients with nodal metastasis and in 54 per cent of those without metastasis this factor was graded slight, or 1 (on a basis of 1 to 4). In a number of patients with metastasis the tumor showed a very slight invasive tendency (Fig. 2*d*), whereas approximately 15 per cent of those not associated with metastasis were strikingly invasive and were graded 3 on this basis. Approximately 40 per cent of primary tumors in both categories were predominantly follicular in architecture, and approximately 12 per cent were predominantly papillary (Fig. 2). A number of each presented an approximately equal mixture of the two components, and 10 per cent of each contained a moderately "solid" component. Some difference in the two groups was noted with respect to the presence or absence of psammoma bodies; thus, these were present in two thirds of the primary tumors associated with nodal metastasis but in only one third of those unassociated with nodal deposits. Psammoma bodies were seen occasionally outside the tumor, scattered throughout the thyroid parenchyma. All such cases were associated with nodal metastasis. No appreciable difference in the degree of sclerosis was

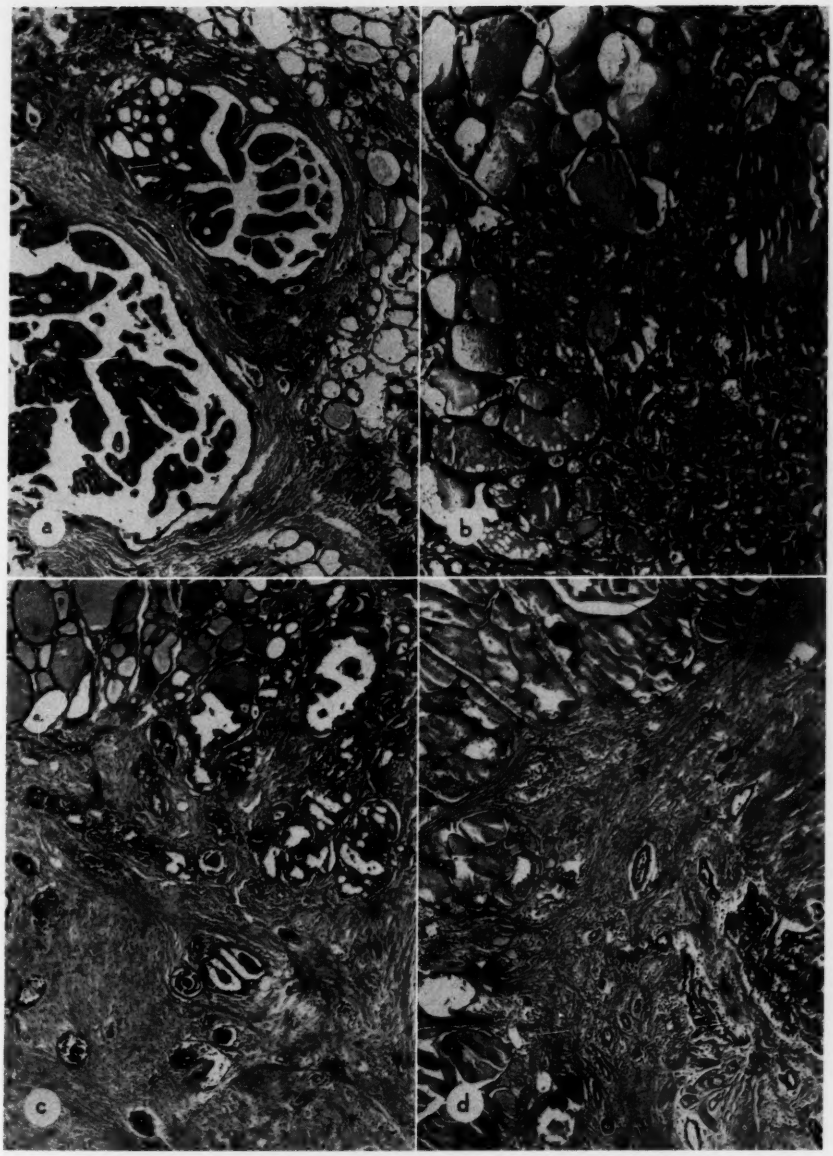

FIG. 2. Occult papillary carcinoma showing variation in architecture and degree of invasiveness. **a.** Small primary carcinoma that is predominantly papillary (×27). **b.** Small primary carcinoma that is predominantly follicular (×50). Almost the entire lesion is shown in both **a.** and **b.** **c.** Margin of a small, somewhat sclerotic primary lesion with moderate invasion of adjacent thyroid parenchyma (×45). **d.** Lesion showing similar degree of sclerosis but very slight invasion (×42). All 4 lesions illustrated were associated with nodal metastasis.

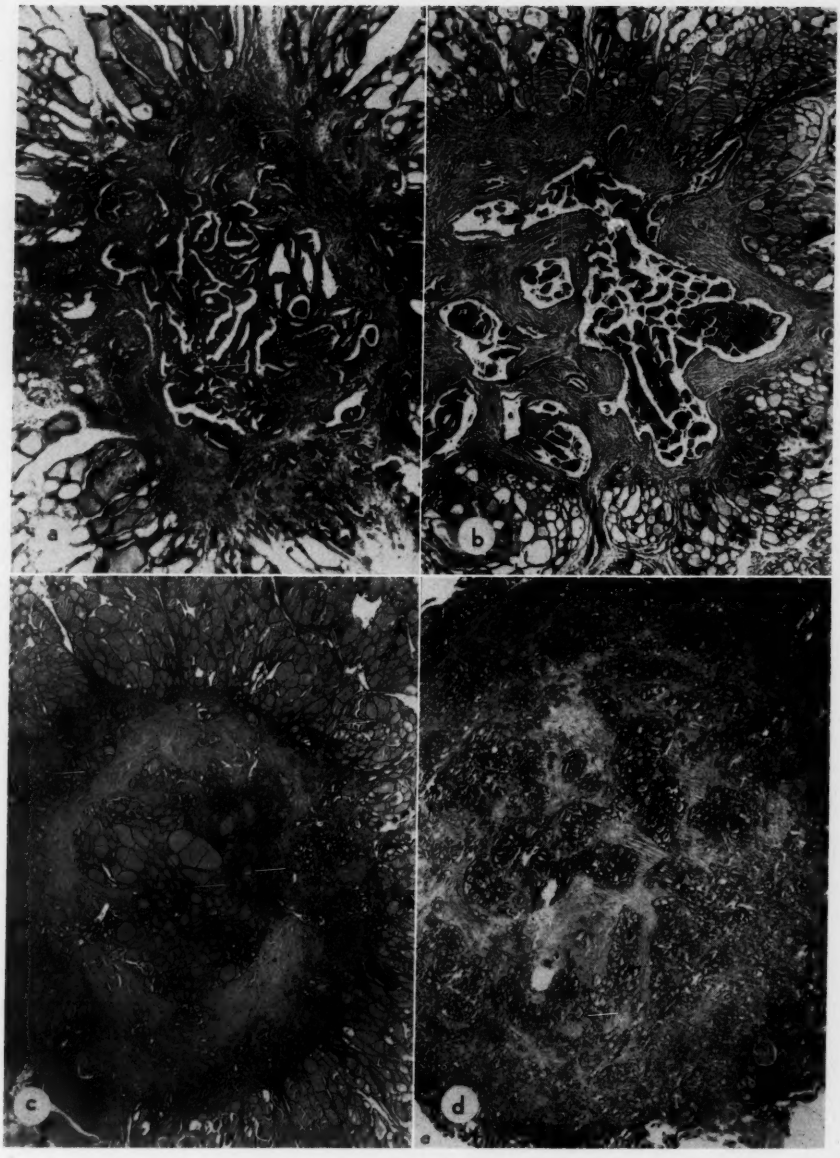

FIG. 3. Occult papillary carcinoma. Comparison of lesions with and without metastasis. Predominantly papillary lesions 3 mm. in diameter associated (**a**) with nodal metastasis (frozen section ×23), and (**b**) without nodal metastasis (×20). **c.** and **d.** Slightly larger, somewhat circumscribed primary lesion showing (**c**) moderate sclerosis associated with nodal metastasis (×8), and (**d**) similar degree of sclerosis and narrow rim of normal thyroid tissue but without nodal metastasis (×13).

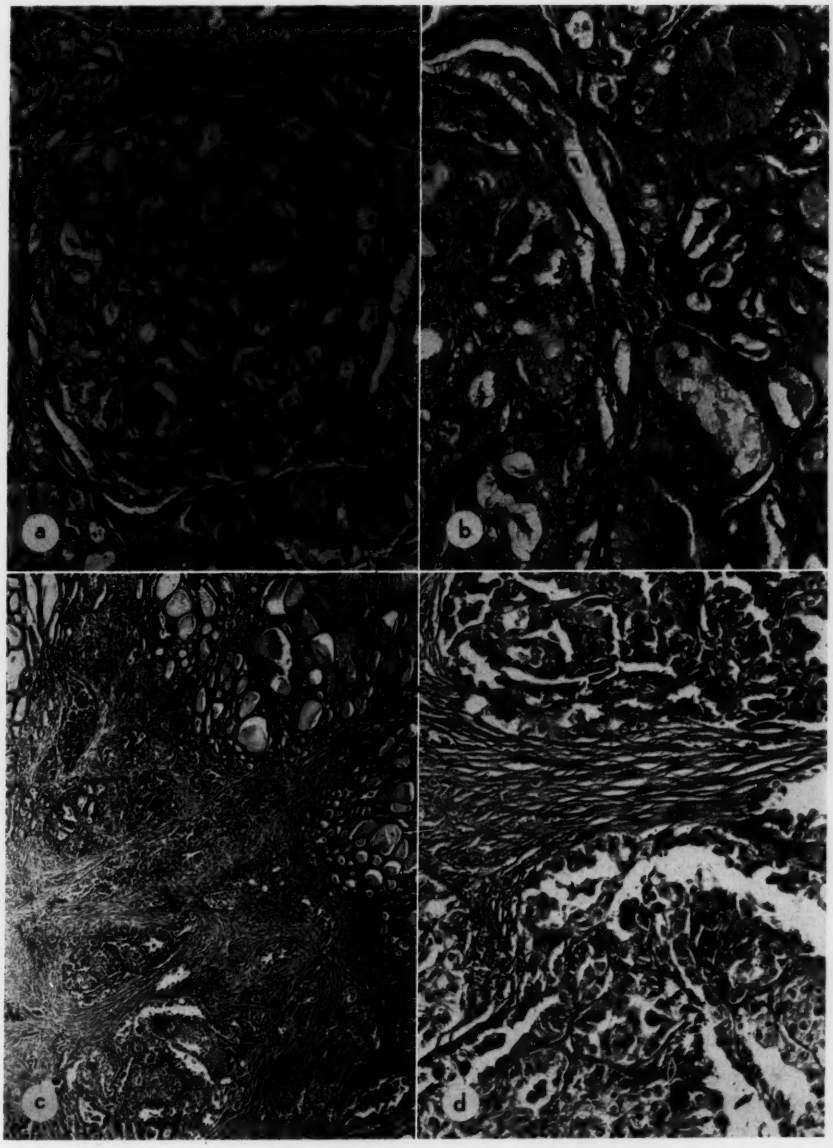

FIG. 4. Occult papillary carcinoma. Comparison of lesions with and without metastasis. Main tumor (**a**) and margin of small papillary carcinoma (**b**) in Graves' disease. Metastasis present in this case. **c** and **d.** Low-power (×33) and high-power (×125) views in another case of Graves' disease with small papillary carcinoma. Metastasis was absent.

seen in the two groups, this factor being graded 3 in approximately 25 per cent of each group. A larger number of patients without metastasis showed a zone of sclerosis immediately surrounding the tumor (Fig. 3*b*). However, a considerable number of patients with metastasis showed a similar sclerotic zone (Fig. 2*d*). The presence of "thyroiditis," that is, an infiltration with plasma cells and lymphocytes within the papillary tumor, was seen only in patients without metastasis.

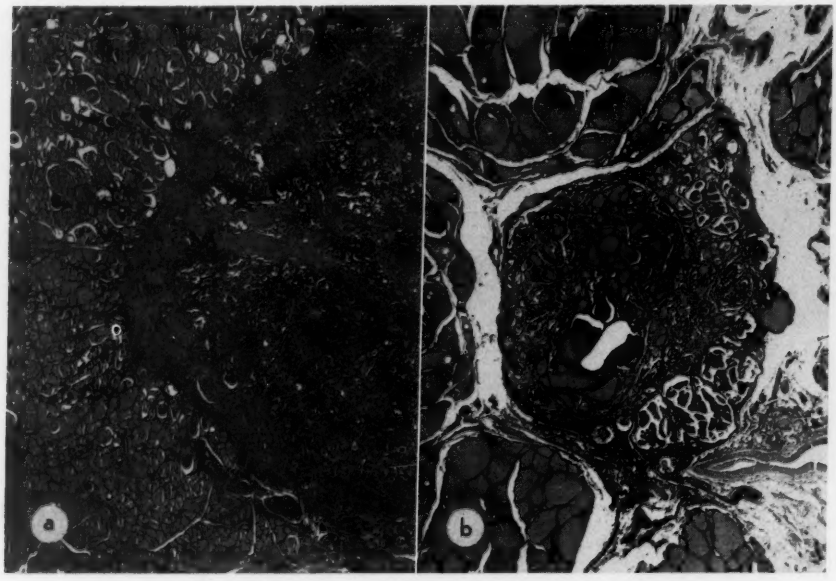

FIG. 5. Bilateral occult papillary carcinoma. **a.** Primary lesion 1 cm. in diameter in right lobe. Lesion removed along with right metastatic nodes in 1944 (×8). **b.** Primary lesion 0.3 cm. in diameter in left lobe. Lesion removed along with left metastatic nodes in 1956 (×15).

In summary, it was observed that there was marked variation in all the factors listed in patients with nodal metastasis and that there was a similar spectrum of architecture, sclerosis and so forth in those without metastasis. It was not considered feasible to attempt a separation of the two on a histologic basis. In Figure 5 are shown 2 lesions removed from the right and left lobes of the thyroid with an interval of twelve years. Neither is particularly impressive as to invasiveness, they vary markedly in degree of sclerosis, and yet both lesions were associated with bulky metastatic growths in the regional nodes.

In almost 50 per cent of cases the nodes sectioned were of predominantly papillary architecture, with psammoma bodies seen in two thirds of the

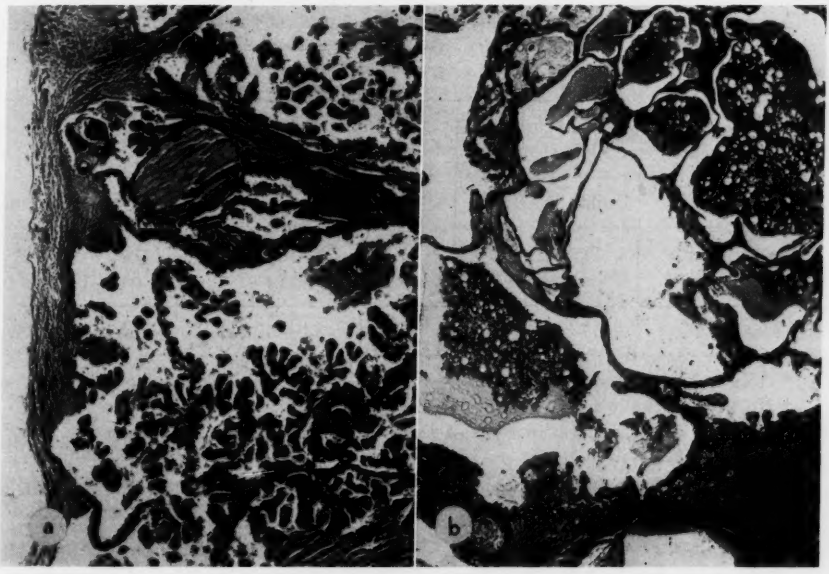

FIG. 6. Occult papillary carcinoma with nodal metastasis. **a.** Common pattern showing marked papillary structure with psammoma bodies (×33). **b.** Uncommon "benign" pattern showing macrofollicular architecture with a few papillary infoldings (×33).

cases (Fig. 6*a*). In a smaller number of cases the nodes were predominantly follicular, and occasionally a macrofollicular pattern was observed, with an unusually benign appearance approaching that of macrofollicular thyroid tissue (Fig. 6*b*). In approximately 10 per cent of cases a solid pattern was prominent.

SURGICAL TREATMENT

The treatment given 82 patients in whom no nodal metastasis was demonstrated is summarized in Table 2. In 52 cases the procedure was subtotal lobectomy, either unilateral or bilateral, in 7 lobectomy, and in 23 double resection (lobectomy on the side of the lesion). The lymph nodes were not dissected out in these cases.

For the patients with enlarged cervical nodes (Table 2), the procedure was total lobectomy in 31, double resection (lobectomy on the side of the lesion) in 10, and total thyroidectomy in 2. Nine patients seen in the earlier years represented by the study underwent unilateral or bilateral subtotal resection. Six patients had no operation on the thyroid and are discussed later. A modified dissection of the neck, with preservation of the jugular vein and sternocleidomastoid muscle, was carried out in 54 patients, 2 of whom underwent bilateral nonsimultaneous procedures. The

TABLE 2. TREATMENT IN 140 CASES OF OCCULT PAPILLARY CARCINOMA OF THYROID

Nodal metastasis	Total cases	Treatment						
		Subtotal lobectomy		Lobectomy	Double resect. and lobect.	Total thyroidectomy	Dissection of neck	
		Unilat.*	Bilat.				Modified	Radical
No	82	11	41	7	23	0	0	0
Yes	58†	6	3	31	10	2	54‡	4
Total	140	17	44	38	33	2	54	4

* Only isthmus removed in 4 cases.
† No thyroid tissue removed in 6 cases.
‡ Bilateral cervical metastatic nodes removed in 2 cases.

extent of nodal dissection varied greatly in these 54 cases; in 4, radical dissection of the neck was performed.

Subsequent surgical procedures. No further surgical procedure on the neck was performed in the 82 patients without nodal involvement. No subsequent nodal enlargement was observed on return visits, nor was any such enlargement mentioned in the follow-up data received by letter.

Seven patients with nodal involvement underwent subsequent removal of nodes at varying intervals after the initial operation (Table 3). Two of these had an apparent second primary lesion on the opposite side removed along with involved nodes on the second side, at intervals of four years and twelve years, respectively. The remaining 5 patients had subsequent removal of affected nodes on the same side. Data on the cases in which the thyroid was not removed are provided later.

COURSE OF DISEASE

Patients with nodal metastasis at operation. Of the 58 patients with nodal metastasis, 53 are living, 4 are dead and 1 is untraced. The 4 deaths occurred at intervals of twenty-four years, ten years, six years and six months, respectively, after surgical treatment and were from causes unrelated to the thyroid. Information on the current status of 52 of the 53 living patients was available either through return visits or letters up to the latter part of 1958 or early 1959, thus providing a maximum of thirty-two years of observation. One patient was living at the end of twenty years, but subsequently has not been traced. Data on 7 patients having further surgical treatment for nodal metastasis during this period of observation are given in Table 3. No known distant metastasis has developed and no patient has known recurrent carcinoma. The duration of observation of the 53 living patients is as follows: thirty years or more, 3 patients; twenty to

TABLE 3. SUBSEQUENT SURGICAL PROCEDURES IN OCCULT PAPILLARY CARCINOMA OF THYROID

Case	Original operation (date)	Subsequent procedures	Alive and well
1	Total rt. lobect., removal of rt. cervical node (1931)	Rt. nodes removed elsewhere (1932)	1958
2	Total rt. and subtotal l. lobect., modif. dissec. of neck on rt. (1942)	L. nodal dissec. and l. thyroid remnant removed (1946). Two l. nodes removed in different months (1947). Rt. post.-triangle node removed (1949)	1958
3	Subtotal rt. lobect., dissec. of post.-triangle nodes (1944)	Total l. lobect. and l. modif. nodal dissec. (1956)	1958
4	Total lobect., modif. dissec. of neck on left (1946)	Node in l. sternomastoid region removed (1947)	1958
5	Total rt. lobect. with dissec. of rt. post.-triangle nodes (1950)	Rt. upper deep cerv. node removed (1958)	1959
6	Total rt. lobect. with modif. dissec. of neck (1951)	Rt. post.-triangle nodes removed (1951). Submental nodes removed (1952). Suprasternal node removed (1955)	1958
7	Total rt. lobect. with modif. dissec. of neck (1952)	Rt. jugular node removed (1953)	1958

twenty-nine years, 4; fifteen to nineteen years, 3; ten to fourteen years, 13; five to nine years, 20; four to five years, 8; and three to four years, 2.

Patients without demonstrable metastasis. Of the 82 patients without nodal metastasis at the time of operation, 71 are living and 11 are dead. The 11 deaths occurred one to thirty years after operation, all of causes unrelated to the thyroid carcinoma. No patients are known to have had either local or distant metastasis. The duration of observation of the 71 living patients is as follows: thirty years or more, 2 patients; twenty-five to twenty-nine years, 3; twenty to twenty-four years, 2; fifteen to nineteen years, 4; ten to fourteen years, 14; five to nine years, 22; and two to four years, 24.

CASES IN WHICH THYROID TISSUE WAS NOT REMOVED AT INITIAL SURGICAL PROCEDURE

Case 1. A 34-year-old woman had noticed an enlarged node in the right side of the neck for three and a half years. The thyroid gland was not palpable on clinical examina-

tion. Operation performed in November 1926 consisted of removal of a right cervical node 4 cm. in diameter which showed metastatic papillary carcinoma of thyroid origin (Fig. 7). The lesion was considered a "lateral aberrant thyroid." The right lobe of the thyroid appeared normal and was not disturbed. Radium treatment was given postoperatively. In June 1955, twenty-eight and a half years later, a total right and subtotal left lobectomy was carried out for nodular goiter. Serial block sections were made. At the capsular area in the right lobe adjacent to the site of previous metastasis a sclerotic region a few millimeters in diameter and containing psammoma bodies was seen, but no more definite evidence of a primary lesion could be located. No associated

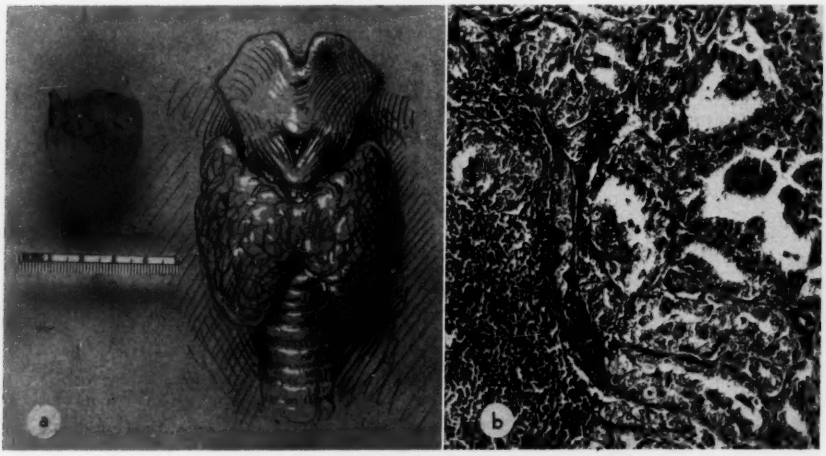

FIG. 7. (Case 1). **a.** Photograph of single metastatically involved right cervical lymph node removed in 1926. Thyroid (drawing) normal and not resected until 1955. **b.** Histologic section of node showing mixed papillary and follicular architecture (×105).

enlargement of lymph nodes was found. The patient was well in August 1958, almost thirty years after initial treatment.

Case 2. A 16-year-old girl had noticed large right cervical nodes for five years. The preoperative clinical impression was tuberculous adenitis. At operation in December 1926, a huge mass of enlarged right cervical nodes was removed (Fig. 8*a*). The thyroid gland was described as normal and was not disturbed. Pathologic examination revealed at least 10 involved nodes, the largest measuring 4 by 2.5 by 2 cm. Microscopically the architecture was that of mixed papillary, follicular and solid carcinoma of thyroid origin (Fig. 8*b*). The patient received postoperative radium therapy. The answer to a follow-up letter in January 1956 indicated that she was alive and well twenty-nine years after operation.

Case 3. A 38-year-old man had noticed enlarged right cervical nodes for four years. The thyroid gland was not palpable. At operation in January 1928, a huge mass of involved nodes was removed from the upper deep jugular region. The thyroid gland was considered normal and was not resected. The patient received postoperative roentgen therapy. He was known to be alive and well twenty years later, but has since been untraced.

Case 4. A 33-year-old woman had noticed enlarged left cervical nodes for seven years The clinical diagnosis was cervical adenitis. The thyroid gland was not palpable. At operation in October 1926, a large mass of nodes extending from the mastoid region to the clavicle was removed. The thyroid appeared normal and was not disturbed. Pathologic examination revealed huge well-encapsulated nodal metastatic masses, the largest measuring 5 by 4 cm. The patient received roentgen therapy to the neck postoperatively. She remained well without further trouble and died of cancer of the breast in 1952, twenty-six years after operation on the thyroid.

Case 5. An 8-year-old girl had had enlargement of cervical nodes bilaterally for four

FIG. 8. (Case 2). **a.** Photograph of huge mass of metastatically involved right cervical lymph nodes removed in 1926. Thyroid (drawing) normal and not resected. **b.** Histologic section showing papillary and follicular architecture of nodal metastatic lesion. The patient was well twenty-nine years later (×75).

months. The thyroid gland was not palpable. The preoperative diagnosis was lymphoma (?). In August 1934, a left posterior cervical node was removed. The original diagnosis was high-grade metastatic carcinoma, later revised to grade 1 papillary and follicular carcinoma of thyroid origin with a prominent solid component (Fig. 9). Because the lesion was considered highly anaplastic, roentgen and radium treatment was instituted. In January 1937, a second cervical lymph node from the left and one from the right were removed. These showed metastatic papillary carcinoma of thyroid origin. In October 1937, a sharply defined area of destruction in the frontal bone and questionable destruction of the body of the second lumbar vertebra were seen. Extensive x-ray and radium treatment was given. The bony defect in the skull gradually filled in and on October 25, 1938, the x-ray appearance of the skull was normal. The patient returned for periodic examinations. When last seen in February 1958, twenty-four years after the original diagnosis, she was well and had no evidence of disease.

Case 6. A 20-year-old man had noticed enlarged nodes in the right cervical region for six years. A preoperative diagnosis of tuberculous adenitis was made. On April 7, 1938, two large right cervical lymph nodes were removed, followed by removal of two addi-

tional involved nodes from the right side a month later. Pathologic examination revealed papillary carcinoma of thyroid origin. The patient returned to his home in South America and has been lost to follow-up.

COMMENT

Although a longer period of observation is necessary in the more recent cases, the data at hand suggest that occult papillary carcinoma, with or without nodal metastasis when first discovered, is an unusually curable dis-

FIG. 9 (Case 5). Histologic section of metastatically involved node removed in 1934. Thyroid was not removed. Patient was free of disease in 1958 (×130).

ease. In no patient of this series was there known development of proved distant metastasis nor residual carcinoma at last report.

There are, in general, two main threats to life in papillary carcinoma of the thyroid, namely, local recurrence in the midline of the neck and distant metastasis, especially to the lungs or bones. The good results with respect to local recurrence in the group under study is understandable by virtue of the ease with which wide removal of the primary tumor may be accomplished. The complete absence of proved distant metastasis in the group, even with extremely bulky nodal spread, was an unexpected but perhaps not too surprising finding, when the low incidence of such spread in papillary thyroid cancer of more conventional type is considered.

The course of the disease in patients in whom no operation on the thyroid was performed provides some grounds for speculation that these tumors may be basically different in their biologic behavior from that of ordinary papillary carcinoma. Five of the 6 patients in this group were traced for periods ranging up to thirty-two years, and no further recurrence or metastasis was demonstrated. Case 1 is particularly interesting in this regard in that the gland was available for study twenty-eight and a half years after the large lymph node was removed, and only a scarred area with psammoma bodies was detected. All 5 patients were given postoperative roentgen or radium therapy. In Cases 1, 3 and 4, current dosage estimates suggest that the amount of irradiation received to the thyroid was not in the therapeutic range. In Cases 2 and 5, effective therapeutic irradiation was administered, according to current assessment of dosage. One must assume in Cases 1, 3 and 4 either that the minimal radium or x-ray treatment given was the effective agent in influencing the course of the disease, or that the carcinoma lacked the potential for further growth and metastasis.

REFERENCES

1. Frantz, V. K.; Forsythe, R.; Hanford, J. M., and Rogers, W. M.: Lateral aberrant thyroids, *Ann. Surg.* **115:** 161 (Feb.) 1942.
2. Black, B. M.: Papillary adenocarcinoma of the thyroid gland, so-called lateral aberrant thyroid tumors, *West. J. Surg.* **56:** 134 (Mar.) 1948.
3. Wozencraft, P.; Foote, F. W., Jr., and Frazell, E. L.: Occult carcinomas of the thyroid: their bearing on the concept of lateral aberrant thyroid cancer, *Cancer* **1:** 574 (Nov.) 1948.
4. Hazard, J. B.; Crile, G., Jr., and Dempsey, W. S.: Nonencapsulated sclerosing tumors of the thyroid, *J. Clin. Endocrinol.* **9:** 1216 (Nov.) 1949.
5. Klinck, G. H., and Winship, T.: Occult sclerosing carcinoma of the thyroid, *Cancer* **8:** 701 (July–Aug.) 1955.
6. Mortensen, JD; Woolner, L. B., and Bennett, W. A.: Gross and microscopic findings in clinically normal thyroid glands, *J. Clin. Endocrinol. & Metab.* **15:** 1270 (Oct.) 1955.
7. Beahrs, O. H., and Woolner, L. B.: The treatment of papillary carcinoma of the thyroid gland, *Surg., Gynec. & Obst.* **108:** 43 (Jan.) 1959.

"METABOLIC INSUFFICIENCY": A DOUBLE-BLIND STUDY USING TRIIODOTHYRONINE, THYROXINE AND A PLACEBO: PSYCHOMETRIC EVALUATION OF THE HYPOMETABOLIC PATIENT*

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ABSTRACT

Triiodothyronine has been recommended in the treatment of "metabolic insufficiency," a syndrome characterized by a low basal metabolic rate, a normal level of serum protein-bound iodine, and a normal thyroidal uptake of radioactive iodine. Only 22 patients fulfilling the foregoing criteria were found in a busy out-patient department during the year of investigation; 18 (11 females and 7 males) were available for detailed study. The average age was 35 years. The most common complaints were nervousness, fatigue and weakness. In 10 patients, the Minnesota Multiphasic Personality Inventory test showed a high degree of abnormality, with responses suggesting depressive, hypochondriacal and schizoid traits. The basal metabolic rate averaged −21 per cent, the serum protein-bound iodine level 5.2 μg. per 100 ml., and the thyroidal I^{131} uptake 23 per cent. Thyroxine (0.1 mg.) triiodothyronine (.025 mg.) and lactose were given in the form of coded tablets which were identical in appearance. Their identity was not disclosed until after completion of the study. Patients assigned to treatment groups by rotation received one tablet daily for one week, two tablets daily during the second week, and three tablets daily for the next two weeks. No significant differences in basal metabolic rates or in symptoms were observed during treatment. Subsequently, the medication in 8 patients was changed, without their knowledge, to the second and then the third drug. The correlation between the type of therapeutic agent used, the basal metabolic rate, and the patient's complaints was poor. Therefore, no support for the specific use of triiodothyronine in the metabolic insufficiency syndrome was demonstrated.

"METABOLIC insufficiency" (1), also known as "non-myxedematous hypometabolism" or "euthyroid hypometabolism" (2, 3), has been characterized as a clinical syndrome associated with a low basal metabolic rate and many symptoms similar to those of hypothyroidism. In this syndrome, unlike hypothyroidism, the serum concentration of protein-bound iodine (PBI) and the thyroidal uptake of radioactive iodine (I^{131}) are within the normal range, and treatment with desiccated thyroid or thyrox-

* Presented at the Annual Meeting of the American Goiter Association, Chicago, Illinois, April 30–May 2, 1959.

ine does not increase the basal metabolic rate significantly or relieve the symptoms.

Recently, renewed interest in this condition has followed the papers of Kurland, Hamolsky and Freedberg (2, 3) wherein they reported 4 patients with "nonmyxedematous hypometabolism." These patients failed to respond by an increase in basal metabolic rate or by improvement in their symptoms of fatigue, lethargy and nervousness, to treatment with as much as 5 grains of desiccated thyroid a day. By using triiodothyronine alone or in combination with desiccated thyroid, these investigators reported striking clinical improvement accompanying a rise in the basal metabolic rate in all 4 patients. Although placebos were used as control medication, a "double-blind" study was not carried out. This would seem indicated in a group of patients with so many symptoms suggestive of neurasthenia.

In surveying our patients with hypometabolism it soon became evident that cases without hypothyroidism were not as common as might be implied by the number of papers reporting favorable effects of triiodothyronine in series as large as 51 cases of this syndrome (1, 4, 5). Moreover, clinical improvement in our patients treated with triiodothyronine was not always obtained nor sustained. Skepticism concerning the effectiveness of triiodothyronine treatment in this syndrome was also supported by a recent editorial by Keating (6) and by several editorials that appeared in *The Lancet* (7, 8) which suggested the need for a "double-blind" study. This paper reports such an investigation, using sodium-*l*-thyroxine, sodium-*l*-triiodothyronine and a lactose placebo.

CLINICAL MATERIAL AND METHODS

Only 22 cases fulfilling the criteria for a diagnosis of "metabolic insufficiency" were found in the busy Washington University clinic population in a twelve-month period, from September 1957 to September 1958. Of these 22 patients, 18 were available for detailed study. The sex distribution was 11 females to 7 males. The ages varied from 16 to 50 years, with an average age of 35. There were 11 Caucasians and 7 Negroes. The average weight for the group was 155 pounds; the weights had been stable in the year prior to the study.

Only those patients were included who had a BMR of −15 per cent or below, and a normal thyroidal I^{131} uptake and serum PBI level. They were further investigated by a questionnaire concerning the usual and more frequent symptoms associated with hypothyroidism, as well as all other pertinent physical and laboratory data. A comparison was made between the percentages of occurrence of symptoms in our hypometabolic group (22 patients) and in Lerman's group with myxedema (77 patients) reported by Means (9).

The frequency of complaints of nervousness and other neurasthenic symptoms suggested that there might be an abnormal psychologic pattern in these cases. In order to investigate this possibility, 10 hypometabolic patients were evaluated by means of

psychometric testing using the Minnesota Multiphasic Personality Inventory test (10). Five were given the test before treatment, and 5 after treatment. This test provides a method of eliciting a patient's personal thinking and feeling by tabulating his responses to a large number of statements reflecting a wide variety of psychiatric disturbances. It includes three scales for assessing the patient's attitudes toward test taking, and nine scales for responses in psychiatric conditions, including hypochondriasis, depression, hysteria, psychopathic deviation, masculinity-femininity, paranoia, psychasthenia, schizophrenia, and manic states.

Most patients in this study were aware that they had a "low metabolism" and that this was related to thyroid function. They were told, therefore, only that they had a "low metabolism" and were being given a special form of "thyroid" to correct it. All patients were seen, treated, and evaluated by the author. The drugs were known by code number only and the medication was not marked in any way. The code was not broken until the completion of the study.

The triiodothyronine was prepared in 0.025-mg. tablets and the thyroxine in 0.1-mg. tablets, these doses being generally accepted physiologic equivalents. All three medications were in the form of small white tablets, identical in appearance. Medications were given as folllows: one tablet a day for one week, then one tablet twice a day during the second week, and finally one tablet three times a day for an additional two weeks. The patients had received no therapy prior to that given in this study. Re-evaluation of the symptoms, BMR, pulse, blood pressure and weight were made at the end of the one-month period.

After treatment for a month or longer, the medication in 8 patients was changed to the second, and subsequently to the third preparation, always in a dosage of one tablet three times a day. At the time of drug change, patients believed they were simply being given an additional supply of medication, and the shift to another drug was made completely without their knowledge. The nature of the new drug was unknown to the investigator. The basal metabolic rate and clinical status were appraised periodically with each form of medication.

RESULTS

The incidence of symptoms in myxedema as tabulated by Lerman (9) and the incidence noted by us in the hypometabolic patients are compared in Table 1. Of the various symptoms considered, only weakness, fatigue, nervousness and constipation occurred in the hypometabolic group with a frequency approximating that in myxedema. Other symptoms such as cold intolerance, dry skin and coarse or falling hair were observed much less often than in myxedema. In contrast, complaints of nervousness were present in 91 per cent of the hypometabolism group as compared with only 35 per cent of the myxedema group.

The clinical characteristics, symptoms, and BMR prior to therapy and after one month's treatment, as well as the other pertinent laboratory data, are recorded in Tables 2, 3 and 4.

In the 18 patients studied the following normal values (average) were found: hemoglobin 13.9 mg. per 100 ml.; serum cholesterol, 219 mg. per 100 ml.; serum PBI 5.3 μg. per 100 ml.; and thyroidal I^{131} uptake 23.5 per

TABLE 1. FREQUENCY OF SYMPTOMS IN HYPOMETABOLISM (OUR SERIES) AND MYXEDEMA (LERMAN'S SERIES*)

Symptom	Per cent of 22 cases of hypometabolism in which symptom occurred	Per cent of 77 cases of myxedema in which symptom occurred
1. Nervousness	91	35
2. Fatigue	82	91
3. Weakness	77	99
4. Constipation	72	61
5. Cold intolerance	41	89
6. Dry skin	13	97
7. Decreased sweating	10	89
8. Coarse hair	5	76

* See Means, J. H. (9).

cent. Only the BMR was abnormal, the average for the 18 patients being −20.7 per cent.

After one month's treatment, the majority of patients in each group reported that they had less weakness and more energy, less fatigue and less nervousness.

To compare the differences in these subjective symptoms before and after treatment with the test medications, values of zero, 1 plus, 2 plus or 3 plus were assigned to the varying degrees of severity of the symptoms. Since these numerical ratings cannot have perfect mathematical relationships with one another, a non-parametric statistic must be used in evaluating them (11). By this analysis, no significant differences in the effects of the drugs could be demonstrated, P being in excess of 0.5 (Table 5).

There was little change in weight and blood pressure. The only significant difference was an increase in the pulse rate with thyroxine (Table 5). Although in most patients there was some improvement in the basal metabolic rate, there was no significant difference between the effects of the three preparations (Table 5).

Only 1 patient had to be dropped from the study because of complaints of increased nervousness. She had received the lactose placebo for one week.

Graphic representations of the effects of the three different drugs in the same patient on the BMR and on the clinical picture are illustrated in Figures 1, 2 and 3. These cases were selected to show that changes in the degree of energy, fatigue and nervousness may not always parallel the changes in BMR, and that administration of a lactose placebo, as well as thyroxine or triiodothyronine, may be associated with an elevation of the BMR and an improvement in the subjective state.

TABLE 2. SYMPTOMS AND SIGNS BEFORE AND AFTER TREATMENT WITH TRIIODOTHYRONINE

Patient	Age (yrs.)	Color	Sex	Hgb. (Gm./100 ml.)	Serum cholesterol (mg./100 ml.)	Serum PBI (γ/100 ml.)	Thyroidal I^{131} uptake (% dose)	Symptoms and Signs											
								Weakness		Fatigue		Nervousness		Weight (lbs.)		Blood pressure (mm.Hg)		Pulse (beats/min.)		B.M.R. (%)	
								Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
L.N.	49	C	F	11.4	287	4.7	17.4	0*	impr.†	+	impr.	+++	+	132	139	110/70	120/80	80	88	−20	−13
G.R.	32	W	M	15.3	256	4.7	18.8	+++	+	++	+	++	+	178	178	140/90	110/80	80	80	−18	−12
C.B.	32	C	F	12.4	187	4.8	35.2	++	0	+++	+	+++	+	161	161	120/88	110/70	82	75	−17	− 7
D.V.	28	W	F	11.8	197	4.2	23.8	0	impr.	0	impr.	0	0	175	167	110/70	105/70	74	75	−28	− 1
M.P.	29	W	M	16.4	284	6.0	25.0	0	0	0	0	++	++	185	184	130/85	120/80	84	84	−21	−11
M.S.	50	W	M	14.6	242	6.1	17.7	+++	+++	+++	+++	+++	++	133	131	122/80	110/70	80	95	−21	−15
Avge.	36.4			12.9	235	5.1	23.6							164	166	122/81	112/75	81	83	−19.6	− 7

* Degree of symptoms: 0 =none; + =mild; ++ =moderate; +++ =severe.
† Improved.

TABLE 3. SYMPTOMS AND SIGNS BEFORE AND AFTER TREATMENT WITH THYROXINE

Patient	Age (yrs.)	Color	Sex	Hgb (Gm./100 ml.)	Serum cholesterol (mg./100 ml.)	Serum PBI (γ/100 ml.)	Thyroidal I^{131} uptake (% dose)	Symptoms and Signs											
								Weakness		Fatigue		Nervousness		Weight (lbs.)		Blood pressure (mm.Hg)		Pulse (beats/min.)		BMR (%)	
								Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
L.R.	35	W	M	15.8	223	4.5	26.0	++	+	+++	++	+	0	139	142	120/85	110/70	64	80	−22	−15
E.S.	38	W	F	12.7	170	6.0	12.8	++	+	++	++	++	++	107	106	110/85	115/80	76	95	−19	− 6
M.H.	17	W	M	16.0	180	4.3	19.5	0	0	0	0	0	0	163	163	106/55	105/70	64	66	−15	−15
F.M.	16	W	M	16.0	—	5.4	23.0	++	++	++	++	++	+	193	189	115/75	110/80	68	75	−33	−26
N.M.	21	C	F	13.2	211	6.0	28.5	+++	0	+++	0	+++	0	117	118	120/80	130/80	80	80	−18	− 9
Z.G.	45	C	F	12.4	252	5.0	—	+	0	0	0	+++	++	149	148	108/70	110/70	88	84	−21	− 7
Avge.	28.5			14.3	207	5.2	20.9							144	144	113/75	113/75	73	80	−21	−15

Degree of symptoms: 0 =none; + =mild; ++ =moderate; +++ =severe.

TABLE 4. SYMPTOMS AND SIGNS BEFORE AND AFTER TREATMENT WITH A LACTOSE PLACEBO

Patient	Age (yrs.)	Color	Sex	Hgb. (Gm./100 ml.)	Serum cholesterol (mg./100 ml.)	Serum PBI (γ/100 ml.)	Thyroidal I^{131} uptake (% dose)	Symptoms and Signs													
								Weakness		Fatigue		Nervousness		Weight (lbs.)		Blood pressure (mm.Hg)		Pulse (beats/min.)		B.M.R. (%)	
								Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
H.S.	39	W	F	15.5	192	4.2	36.2	+++	+++	+++	+++	++	++	167	167	110/70	120/80	72	80	−23	−21
C.A.	39	W	F	15.7	—	6.2	35.0	+++	0	+++	++	+++	+	125	126	115/70	120/82	84	88	−15	−14
A.B.	44	C	F	14.0	261	5.2	14.5	++	++	++	++	++	++	168	165	130/80	125/80	80	60	−24	−11
A.W.	37	W	M	15.0	205	6.0	30.0	++	+	++	++	++	++	163	160	110/80	120/80	70	62	−29	−16
L.M.	46	C	F	14.6	201	6.8	13.7	+++	+	+++	+	+++	++	113	112	110/70	110/60	88	70	−19	−17
M.C.	34	C	F	13.7	214	4.3	26.0	+++	0	+++	0	+++	+	199	193	110/80	100/65	88	80	−20	−17
Avge.	39.6			14.7	214	5.5	25.9							156	154	114/75	117/75	80	73	−216	−16

Degree of symptoms: 0 = none; + = mild; ++ = moderate; +++ = severe.

TABLE 5. STATISTICAL ANALYSIS

Variable	Thyroxine *vs.* placebo	Thyroxine *vs.* triiodothyronine	Triiodothyronine *vs.* placebo
	P value	P value	P value
BMR difference	<.40	<.60	<.30
PULSE difference	<.05	<.05	<.10
WEIGHT difference	<.40	<.90	<.70
BLOOD PRESSURE			
systolic	<.80	<.20	<.10
diastolic	—	<.60	<.50
WEAKNESS difference	in excess	in excess	in excess
FATIGUE difference	of	of	of
NERVOUSNESS difference	.50	.50	.50

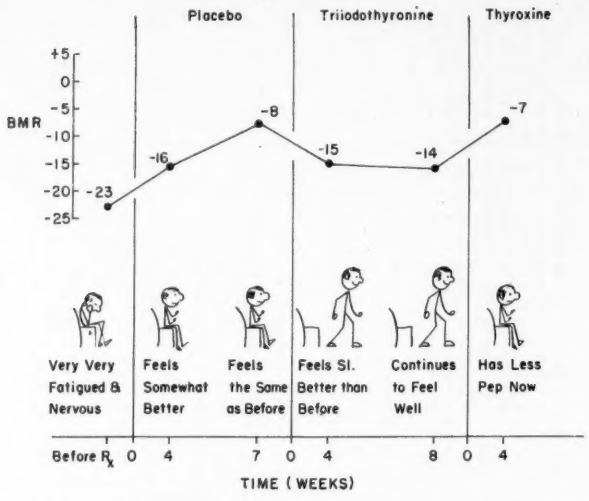

FIG. 1. *Patient A.W.* (male). Effects of triiodothyronine, thyroxine and a lactose placebo, given by the "double-blind" technique, on the BMR and clinical state.

With the Personality Inventory test, these patients had scores high in scales of hypochondriacal, depressive and schizoid traits. That is, they had responses in these categories similar to those which would be made by patients with known psychiatric diagnoses. The responses toward test-taking

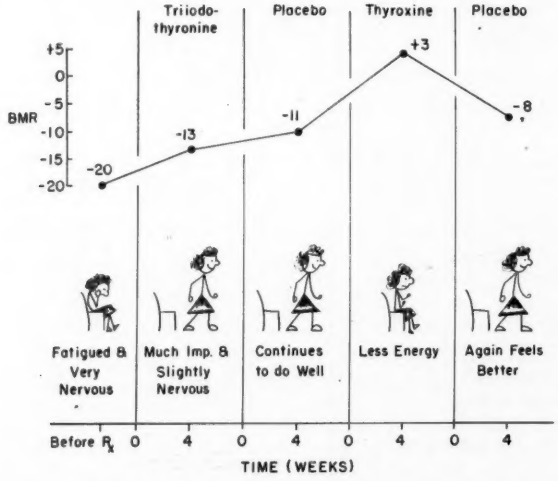

FIG. 2. *Patient L.N.* (female). Effects of triiodothyronine, thyroxine and a lactose placebo, given by the "double-blind" technique, on the BMR and clinical state.

itself were normal. The observed pattern indicated no specific psychiatric diagnosis, but did show signs of general psychiatric maladjustment.

Despite the fact that many patients showed some increase in the BMR and definite clinical improvement at some time during the course of study, the personality profile remained virtually unchanged after treatment.

DISCUSSION

Vigorous advertising and several papers reporting large numbers of patients with the symptoms of metabolic insufficiency have implied that this

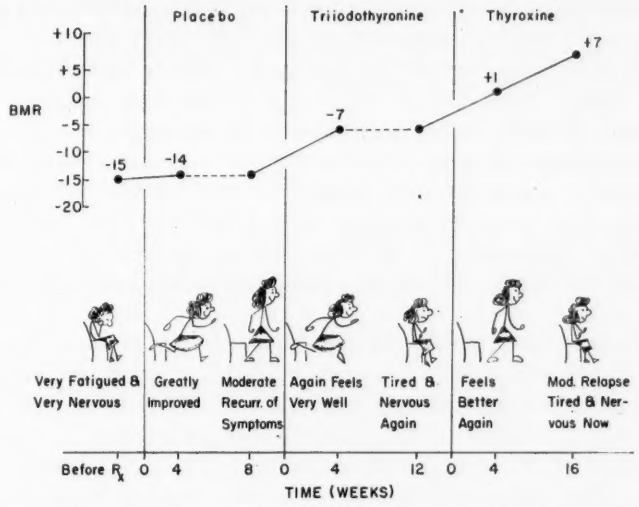

FIG. 3. *Patient C.A.* (female). Effects of triiodothyronine, thyroxine, and a lactose placebo, given by the "double-blind" technique, on the BMR and clinical state.

is a common condition. Thus, Fields (4) reported 40 cases of "metabolic insufficiency." However, the basis for the diagnosis was unclear, as the diagnostic criteria employed were not enumerated. Morton (5) reported 51 patients with "metabolic insufficiency," 46 of whom were said to have been relieved by triiodothyronine, although no controls such as placebo medication or double-blind technique were used. All of his patients, however, could not be classified as hypometabolic, as some had basal metabolic rates as high as +2 per cent. Moreover, Freedberg *et al.*, (who have been largely responsible for renewal of interest in this syndrome) in the past few years could find only 15 patients with the condition as they define it (12).

The 22 hypometabolic patients reported here were found during a one-year period in a university clinic in which 10,141 new patients were seen

during the year. Of these, 2,290 were new medical patients. Thus the 22 patients who fulfilled all of the necessary criteria for a diagnosis of "metabolic insufficiency" represented approximately a 1 per cent incidence of the syndrome among the medical patients seen in this clinic.

This investigation has made no attempt to define the syndrome of "metabolic insufficiency" or "euthyroid hypometabolism" on a physiologic basis. It has been suggested that in these patients there is a defect in the penetration or activation of thyroxine in the cells, which is circumvented by triiodothyronine (3). However, to date, there has been no biochemical evidence that thyroxine degradation is abnormal. Although the fractional thyroxine turnover rate is slower in many of these hypometabolic patients, the amount of thyroxine degraded per day is normal (13).

Certain of the data compiled in this study indicate that there is no end-organ or cellular deficiency of active thyroid hormone in these patients. If there were, the clinical picture should resemble myxedema more closely. Despite careful questioning and detailed observation, we could find only a few patients in this group with complaints and physical signs compatible with a true decrease in cellular function, *e.g.*, dry hair, dry skin, decreased sweating, or a slow pulse. In addition, anemia, an elevated serum cholesterol level, and laboratory findings common in thyroid deficiency, were not present.

The abnormal personality patterns shown by these patients raises the question of the relationship between the BMR, clinical picture, and psychologic state. One possibility is that the low metabolic rate accounts for the abnormal personality, as it is known that a variety of abnormal emotional states are associated with myxedema (14, 15). However, the fact that even after an increase in the BMR and improvement in the clinical state, the personality profile remained unchanged, suggesting that the low metabolic rate is probably the effect of, and not the cause of the abnormal psychologic state. The exact relationship between the emotional state and the BMR is worthy of further investigation.

No support or specific indication for the use of triiodothyronine in this syndrome was demonstrated in this study. Further, the greater degree of improvement occasionally obtained with a lactose placebo as compared to the biologically active compounds, thyroxine and triiodothyronine, in the same patient is additional confirmation of the lack of a significant difference between the effects of these preparations in this particular syndrome.

It is concluded that the term "metabolic insufficiency" is vague and misleading and might well be dropped. In patients with the syndrome indicated by this diagnosis there is probably no discrete biochemical lesion, but instead, an underlying psychologic disorder not directly involving the thyroid.

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REFERENCES

1. TITTLE, C. R.: Effects of 3, 5, 3′-*l*-triiodothyronine in patients with metabolic insufficiency, *J.A.M.A.* **162:** 271 (Sept. 22) 1956.
2. FREEDBERG, A. S.; KURLAND, G. S., and HAMOLSKY, M. W.: Effect of L-triiodothyronine alone and combined with L-thyroxine in nonmyxedematous hypometabolism: preliminary report, *New England J. Med.* **253:** 57 (July 14) 1955.
3. KURLAND, G. S.; HAMOLSKY, M. W., and FREEDBERG, A. S.: Studies in non-myxedematous hypometabolism. I. The clinical syndrome and the effect of triiodothyronine, alone or combined with thyroxine, *J. Clin. Endocrinol. & Metab.* **15:** 1354 (Nov.) 1955.
4. FIELDS, E. M.: Treatment of metabolic insufficiency and hypothyroidism with sodium liothyronine: preliminary report, *J.A.M.A.* **163:** 817 (Mar. 9) 1957.
5. MORTON, J. H.: Sodium liothyronine in metabolic insufficiency syndrome and associated disorders: preliminary report, *J.A.M.A.* **165:** 124 (Sept. 14) 1957.
6. KEATING, F. R., JR.: (Editorial) "Metabolic insufficiency," *J. Clin. Endocrinol. & Metab.* **18:** 531 (May) 1958.
7. EDITORIAL: Triiodothyronine for "metabolic insufficiency," *Lancet* **2:** 826 (Oct. 20) 1956.
8. ANNOTATIONS: "Metabolic insufficiency," *Lancet* **2:** 992, 1957.
9. MEANS, J. H.: The Thyroid and Its Diseases, ed. 2. Philadelphia, J. B. Lippincott Co., 1948, p. 233.
10. MCKINLEY, J. C., and HATHAWAY, S. R.: A multiphasic personality schedule (Minnesota). 11 A. A differential study of hypochondriasis, *J. Psychol.* **10:** 255 (Oct.) 1940.
11. WALKER, H., and LEV, J.: Statistical Inference. New York, Henry Holt and Co., 1953, p. 435.
12. FREEDBERG, A. S.: Personal communication quoted by Keating, F. R., Jr., ref. (6).
13. KURLAND, G. S.; BUSTOS, J. G.; HAMOLSKY, M. W., and FREEDBERG, A. S.: Studies in nonmyxedematous hypometabolism. II. Turnover of I^{131}-labeled thyroxine after intravenous infusion, *J. Clin. Endocrinol. & Metab.* **17:** 1365 (Nov.) 1957.
14. ASHER, R.: Myxoedematous madness, *Brit. M. J.* **2:** 555 (Sept. 10) 1949.
15. CARPELAN, H.: Mental disorders, in thyroidectomized patients, *Acta psychiat. et neurol. scandinav.* Suppl. 116, 1957.

DEPRESSION OF THE SERUM CHOLESTEROL LEVEL IN MYXEDEMATOUS PATIENTS BY AN ORAL DOSAGE OF SODIUM DEXTRO-THYROXINE WHICH HAS NO EFFECT ON THE BASAL METABOLIC RATE OR ELECTROCARDIOGRAM*†

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ABSTRACT

In the previous studies, it was found that high dosage (10 mg. daily) of sodium dextro-thyroxine raised the metabolic rate and lowered the serum cholesterol level without an initial effect on the electrocardiogram. This was taken as pharmacologic evidence that the dextro-thyroxine was actually free of contamination by the levo-isomer. From 1 to 2 mg. a day, in 3 successive cases of untreated myxedema lowered the serum cholesterol level in three or four weeks from 500 to 300, 330 to 215 and 375 to 280 mg. per 100 ml., without raising the basal metabolic rates, which were, at the end of the study, respectively, −36, −40 and −40 per cent. The electrocardiograms also remained unchanged. These results suggest that the metabolic pathway by which dextro-thyroxine or its products produces the reduction in serum cholesterol concentration is independent of that by which it raises the BMR or potentiates the ECG.

RESULTS of administration of sodium dextro-thyroxine[1] in a dosage of 10 mg. daily to a myxedematous patient has been reported previously (1). In this patient, the basal metabolic rate rose from −35 to −15 per cent, and the serum cholesterol concentration fell from 600 mg. to 282 mg. per 100 ml. in eighteen days. There was no effect on the electrocardiogram, yet the dosage was about 100-fold that of the levo-thyroxine commonly used in the treatment of myxedema. This seemed to indicate that sodium dextro-thyroxine was free of contamination by the levo-isomer. The question then arose as to whether or not the reduction of the serum cholesterol level by dextro-thyroxine was necessarily associated with an

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[1] Generous supplies of sodium dextro-thyroxine were provided by the Baxter Laboratories, Morton Grove, Illinois.

increase in the basal metabolic rate. We have been able to show in the following 3 patients that this is not the case.

CASE ABSTRACTS

Case 1

Mrs. Rosario V., a 46-year-old Mexican housewife, had spontaneous untreated myxedema. After several trials, the basal metabolic rate was found to be −35 per cent, the serum protein-bound iodine (PBI) level 1.5 μg. per 100 ml., and the serum cholesterol level 500 mg. per 100 ml. On September 25, 1958, a daily oral dose of 1 mg. of sodium dextro-thyroxine was prescribed. On October 28, the BMR was still −36 per cent, but the serum cholesterol level was 310 mg. per 100 ml. (Fig. 1). There was no effect on the electrocardiogram at this time, after thirty-three days of medication (Fig. 2). After

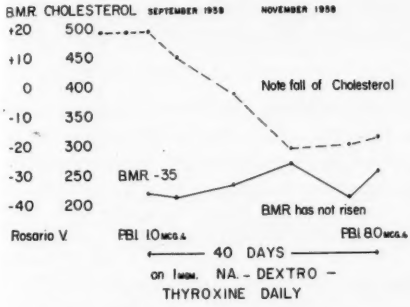

FIG. 1. Effect of 1 mg. of sodium dextro-thyroxine daily in Case 1. Note the rapid reduction of serum cholesterol concentration without a rise in the BMR.

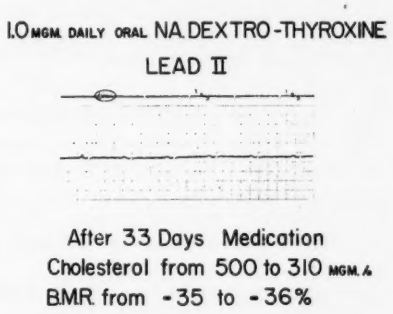

FIG. 2. ECG, Lead II, in Case 1 after thirty-three days of treatment with 1 mg. daily of sodium dextro-thyroxine by mouth.

eighty-two days of medication, the dose having been increased to 3 mg. daily, the BMR was −20 per cent, the serum cholesterol level had decreased further to 230 mg. per 100 ml., and the ECG showed small T waves.

Case 2

Mr. Myron B., a white 59-year-old male with spontaneous untreated myxedema, had a basal metabolic rate of −40 per cent, a serum PBI level of 1.5 μg. per 100 ml., and a serum cholesterol level of 330 mg. per 100 ml. On December 18, 1958, sodium dextro-thyroxine was started in a dosage of 2 mg. daily by mouth. After thirty days of this medication the basal metabolic rate was still −40 per cent, and the serum cholesterol concentration was 215 mg. per 100 ml. (Fig. 3). At this time the electrocardiogram showed no potentiation (Fig. 4).

Case 3

Mrs. Sidonia E. (Fig. 5), a 79-year-old German woman, had almost complete hypothyroidism. The serum PBI level ws 2.0 μg. per 100 ml. and the thyroidal I^{131} uptake was 12.5 per cent. The ECG was not completely flat, and her mental and physical condition was not myxedematous, nevertheless the BMR on three occasions was −40

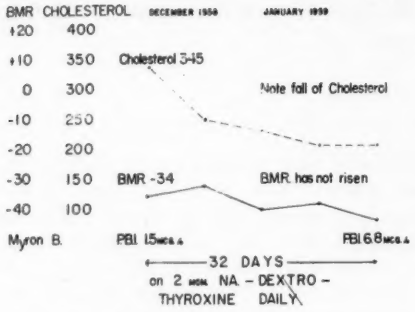
BMR CHOLESTEROL DECEMBER 1958 JANUARY 1959
+20 400
+10 350 Cholesterol 345
0 300 Note fall of Cholesterol
-10 250
-20 200
-30 150 BMR -34 BMR has not risen
-40 100
Myron B. PBI 15 mcg.% PBI 6.8 mcg.%
32 DAYS
on 2 mgm. NA - DEXTRO - THYROXINE DAILY

FIG. 3. Effect of 2 mg. of sodium dextro-thyroxine daily in Case 2. Note the rapid reduction of serum cholesterol concentration without a rise in the BMR.

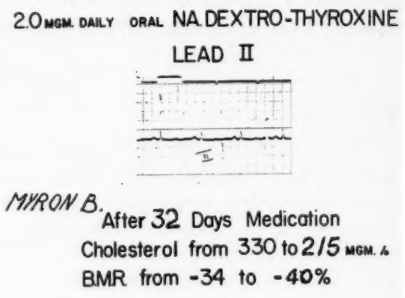
2.0 MGM. DAILY ORAL NA DEXTRO-THYROXINE
LEAD II
MYRON B.
After 32 Days Medication
Cholesterol from 330 to 215 MGM. %
BMR from -34 to -40%

FIG. 4. ECG, Lead II, in Case 2 after thirty days of treatment with 2 mg. daily of sodium dextro-thyroxine by mouth.

per cent and the serum cholesterol level was about 375 mg. per 100 ml. During treatment with 2 mg. of sodium dextro-thyroxine daily the serum PBI level rose to over 20 μg. per 100 ml. and the serum cholesterol level fell in six weeks to 280 mg. per 100 ml., but the BMR was still −40 per cent. An apparent temporary rise to −20 per cent at three weeks may have been false or, if actual, due to the temporary summation of small amounts of levo-thyroxine in the tissues along with the dextro-thyroxine.

DISCUSSION

The possibility that a dose of levo-thyroxine may yet be found that is not calorigenic in myxedema and that will lower the serum cholesterol level without raising the BMR, cannot be excluded. However, this may be difficult, since the basal metabolic rate in myxedema is easily increased by small doses of levo-thyroxine. In the euthyroid person, on the other hand, whose tissues contain a normal amount of levo-thyroxine, a non-calorigenic dose may be easier to achieve. Indeed, Strisower *et al.* (2) have demonstrated significant lowering of serum lipoprotein and cholesterol levels in euthyroid schizophrenic patients by administration of desiccated thyroid.

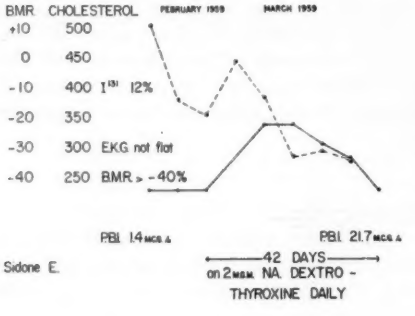
BMR CHOLESTEROL FEBRUARY 1959 MARCH 1959
+10 500
0 450
-10 400 I131 12%
-20 350
-30 300 E.K.G. not flat
-40 250 BMR > -40%
PBI 1.4 mcg.% PBI 21.7 mcg.%
Sidone E.
42 DAYS
on 2 mgm. NA. DEXTRO - THYROXINE DAILY

FIG. 5. Fall of serum cholesterol level and transient rise in BMR in Case 3 during treatment with 2 mg. daily of sodium dextro-thyroxine by mouth.

The basal metabolic rate was not determined, but there was only a slight increase in the pulse rate, and no loss of weight.

Strisower and associates (2) stated that "it is possible that the control of metabolism and the control of lipoproteins are two separate functions of the thyroid gland." Goolden (3) reported that tetraiodothyroacetic acid given to a euthyroid person lowered the plasma cholesterol level without causing loss of weight, and Trotter (4), reported that triiodothyroacetic acid lowered blood cholesterol concentration without significantly altering the basal metabolic rate. Bauer, McGavack and Swell (5) observed depression of the serum cholesterol level in 4 euthyroid patients, but no evidence of metabolic stimulation, during administration of triiodothyropropionic acid.

Dextro-thyroxine may affect the concentration of serum cholesterol by acting through one of its deaminated and deiodinated analogues. Since the molecule is not optically active after deamination, such an analogue may originate from either isomer. The minimal calorigenic activity of the dextro-thyroxine, as such, permits the administration of relatively enormous amounts to serve as a substrate for the deaminating enzymes. However, it is also quite possible that dextro-thyroxine acts directly without degradation. Further attempts should be made to follow this metabolic pathway.

These studies indicate that the enzyme systems or metabolic pathways by which dextro-thyroxine or its products (and hence, probably levothyroxine or its products) effect the reduction of serum cholesterol concentration are independent of those by which they raise the basal metabolic rate and alter the electrocardiogram.

REFERENCES

1. Starr, P.: Comparative clinical and biochemical effects of the dextro- and levo-isomers of thyroxine, *A.M.A. Arch. Int. Med.* **101**: 722–730 (Apr.) 1958.
2. Strisower, B.; Gofman, J. W.; Galioni, E. F.; Rubinger, J. H.; Pouteau, J., and Guzvich, P.: Long-term effect of dried thyroid on serum-lipoprotein and serum-cholesterol levels, *Lancet* **1**: 120–123 (Jan. 19) 1957.
3. Goolden, A. W. G.: The physiological activity of tetraiodothyroacetic acid, *Lancet* **1**: 890–891 (June 9) 1956.
4. Trotter, W. R.: The effect of triiodothyroacetic acid on blood-cholesterol levels, *Lancet* **1**: 885–889 (June 9) 1956.
5. Bauer, H. G.; McGavack, T. H., and Swell, L.: Depression of the serum cholesterol level by triiodothyropropionic acid, *J. Clin. Endocrinol. & Metab.* **19**: 490–493 (Apr.) 1959.

OBJECTIVE PSYCHOLOGIC ASSESSMENT OF THE THYROTOXIC PATIENT AND THE RESPONSE TO TREATMENT: PRELIMINARY REPORT*

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ABSTRACT

Heretofore, the role of the personality in thyrotoxicosis has been stressed, and frustration of needs proposed as the predisposing factor in the onset of the disease. Our observations in 10 thyrotoxic patients and controls indicate that the role of personality in thyrotoxicosis has been overstressed. Three psychologic tests were used to obtain an objective measurement of the emotional responses associated with thyrotoxicosis, to compare these findings with those in normal subjects and other groups, and to observe the effect of treatment. A description of the techniques used and an analysis of the data obtained are presented. The results led to the following conclusions: 1) There is a definite relationship between the clinical and psychologic assessments of thyrotoxicosis. 2) With objective psychologic techniques, the performance of untreated thyrotoxic patients is significantly different from that of normal controls, patients with somatization, and patients with obsessive-compulsive and schizophrenic reactions but strongly similar to that of patients with organic structural changes in the brain. 3) Thyrotoxicosis is a metabolic disease manifested by impaired psychobiologic integration, involving particularly the autonomic nervous system. The adequately treated thyrotoxic patient closely resembles the normal person, who when under stress tends to internalize his conflicts and frustrations.

THE association of disturbed emotions with thyrotoxicosis is well recognized. Frequently, emotional factors are a precipitating cause of the disease, and the clinical picture is one of hyperactivity, emotional lability and hypersensivity. The patient is tense, jittery and restless; he sits forward anxiously on the edge of his chair; his mood is labile, and tears are caused by trivialities; his speech is often rapid and excited; and he may

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experience forebodings of impending disaster. Even the occasional depressed patient is apt to be agitated and markedly restless.

The problem, however, is whether thyrotoxicosis represents a characteristic response of a special personality type to a specific psychic insult. Previous studies have been made by means of psychiatric interviews stressing the importance of emotional factors in the etiology of the disease. Particular reference has been made to frustration of dependent needs (1).

In this study, we tested the hypothesis that it is possible with objective psychologic techniques to measure the emotional responses associated with thyrotoxicosis. We compared findings in thyrotoxic patients with those in normal subjects and other groups, and observed the effect of treatment. The data obtained lead us to the conclusion that the role of personality in thyrotoxicosis may have been overstressed.

SUBJECTS AND METHODS

Subjects

Seven female and 3 male thyrotoxic patients were studied. One of them also exhibited the schizophrenic reaction, 1 neurosis, and 1 the changes associated with psychobiologic decline (2). Nine had diffuse toxic goiter and 1 had nodular toxic goiter. The mean age was 42 years, with a standard deviation of 9 years. The patients were from the middle socio-economic group and had completed the equivalent of a twelfth grade education. The clinical diagnosis of thyrotoxicosis was confirmed in each instance by at least three of these laboratory tests: thyroidal uptake and turnover of I^{131}, chemical determination of serum protein-bound iodine concentration, *in vitro* erythrocyte uptake of I^{131}-labeled *l*-triiodothyronine, PBI^{131} conversion ratio and basal metabolic rate. The patients were otherwise unselected.

The thyrotoxic group was matched for age, sex, socio-economic status, and educational background with normal controls, patients with somatization, obsessive-compulsive and schizophrenic reactions, and those with known structural and/or physiologic changes in the brain (3 with avitaminosis, 3 with cerebral arteriosclerosis, 3 with post-traumatic encephalopathy, and 1 recovered from a cerebrovascular accident). It was not possible to match one patient, 72 years of age, with a schizophrenic.

Method of study

The psychologic examination was carried out in each thyrotoxic patient before treatment and during the response to treatment. One patient was treated with antithyroid drugs and surgery, 2 with antithyroid drugs and radioiodine, and 7 with radioiodine alone.

Thirty sets of observations made on these 10 patients in the internist's office were delivered for scoring to the consulting psychologist, who knew nothing of the patients. The psychologist and internist independently classified the degree of toxicity in each case. The clinical ratings were correlated with those based on the psychologic examinations. The results of the tests on the thyrotoxic patients were compared with those on the other groups.

Tests

The following 3 psychologic tests were used. The first and the second assessed neuroticism, as defined by Eysenck (3); the third discriminated between normal subjects, psychotic subjects, and patients with structural brain disease.

1. *Maudsley Medical Questionnaire*—C. Modified for the American population, this is a 40-item neuroticism inventory, constructed through a series of item analyses and validation experiments. Results are correlated highly with psychiatric opinion on neurotic and normal subjects. The score is the number of yes answers (C) to certain questions, *e.g.*, "Do you get palpitations or thumping of your heart?" and "Do you sweat a great deal without exercise?"

2. *Crown Word Connection List*—N. Modified for the American population, this is a controlled association test in which each item consists of a stimulus word and two possible response words, one of which is an "abnormal" response and the other a "normal" response. The score is the number of abnormal responses (N) to stimulus words, *e.g.*, SINK: wash, drown.

TABLE 1. INTERCORRELATIONS OF PSYCHOLOGIC TESTS

	C*	N	S	E	A
C	1.00				
N	0.08				
S	0.03	0.10			
E	0.10	0.02	0.06		
A	0.08	0.23	0.16	0.11	

* C = Score of Maudsley Medical Questionnaire.
N = Score of Crown Word Connection List.
S, E and A = Scores of Color Naming Stress Test.

3. *Color Naming Stress Test*—S, E and A. This is a visual technique, evaluating the ability to hold two concepts in consciousness simultaneously. Names of colors are printed in colored inks—not, however, in the color represented by the written word. For example, the word "blue" may be printed in red ink. The subject is asked to name the color of the ink, disregarding what the word spells. Three scores are derived: the time, in seconds, required to call off the color of the ink (S score); the errors in performance (E score); and the level of aspiration (A score), when the subject is advised of his time and asked to estimate the number of seconds it would take him to do the task again.

The tests for the psychologic examination appear to be uncorrelated, measuring independent dimensions of the personality. The intercorrelations of the several tests are shown in Table 1. The test-retest reliability for 100 normal controls is shown in Table 2.

RESULTS

There is a significant association between the psychologic and clinical assessments of toxicity (r was calculated to be +0.56, significant at the 1 per cent level). From this association it may be argued that it is possible to assess toxicity by objective psychologic techniques.

TABLE 2. TEST RELIABILITY FOR 100 NORMAL CONTROLS

Test	*r*
C	0.73
N	0.68
S	0.78
E	0.72
A	0.69

The performances of thyrotoxic patients before and after adequate therapy were compared by calculating *t*, using the formula for independent means; they were significantly different, as shown in Table 3.

Qualitatively, the findings revealed that the thyrotoxic patient prior to treatment tends to worry about humiliating experiences and is troubled with feelings of inferiority and self-consciousness. He is emotionally labile. When he must consider more than one thing at a time, his problem-solving time increases and he makes errors. He tends to set goals for himself which are unrealistic. He tends to internalize his conflicts and frustrations.

The performance of untreated thyrotoxic patients was compared with that of other groups, and tested by calculating *t*. The values are shown in Tables 4–8. It is likely that untreated thyrotoxic patients come from a different universe of patients than do normal controls and patients with somatization, obsessive-compulsive reactions, and probably schizophrenic reactions. Untreated thyrotoxic patients do not differ significantly from patients with structural and/or physiologic changes in the brain.

The performance of adequately treated thyrotoxic patients was similarly compared with that of the other groups. The *t* values are shown in Tables 9–13. It is likely that adequately treated thyrotoxic patients come from a different universe of patients than do patients with schizophrenic reactions and known structural and/or physiologic changes in the brain. Ade-

TABLE 3. PERFORMANCES OF THYROTOXIC PATIENTS BEFORE AND AFTER ADEQUATE THERAPY

Test	Before adequate therapy	After adequate therapy	*t*	*P*
C	15.5	9.6	8.78	.001
N	7.6	6.0	2.40	.05
S	2.4	1.9	4.51	.01
E	6.7	1.8	4.05	.01
A	1.30	1.14	3.12	.05

TABLE 4. PERFORMANCE OF UNTREATED THYROTOXIC PATIENTS COMPARED WITH THAT OF CONTROLS

Test	Untreated thyrotoxic patients	Normal controls	*t*	*P*
C	15.5	2.9	4.54	.001
N	7.6	3.8	2.56	.05
S	2.4	1.8	3.19	.01
E	4.7	1.1	2.58	.05
A	1.30	1.15	1.69	n.s.*

* Not significant.

quately treated thyrotoxic patients do not differ significantly from normal controls and patients with somatization and obsessive-compulsive reactions.

It appears likely, therefore, from the findings that: 1) there is no significant difference between the performances measured by the foregoing 3 objective psychologic techniques, of untreated thyrotoxic patients and patients with known structural and/or physiologic changes in the brain, and 2) thyrotoxicosis is a metabolic disease manifested by impaired psychobiologic integration, involving particularly the autonomic nervous system.

It also appears likely from the findings that there is no significant difference between the performance, using the 3 objective psychologic techniques described, of normal controls, patients with somatization and obsessive-compulsive reactions, and adequately treated thyrotoxic patients; moreover, adequately treated thyrotoxic patients closely resemble normal persons, who when under stress tend to internalize their conflicts and frustrations. When the performances of the treated thyrotoxic patients are

TABLE 5. PERFORMANCE OF UNTREATED THYROTOXIC PATIENTS COMPARED WITH THAT OF PATIENTS EXHIBITING SOMATIZATION REACTIONS

Test	Patients with untreated thyrotoxicosis	Patients with somatization reactions	*t*	*P*
C	15.5	16.9	0.42	n.s.
N	7.6	4.5	0.54	n.s.
S	2.4	2.0	0.62	n.s.
E	4.7	2.1	2.22	.05
A	1.30	1.06	2.64	.05

TABLE 6. PERFORMANCE OF UNTREATED THYROTOXIC PATIENTS COMPARED WITH PATIENTS EXHIBITING OBSESSIVE COMPULSIVE REACTIONS

Test	Patients with untreated thyrotoxicosis	Patients with obsessive compulsive reactions	*t*	*P*
C	15.5	22.1	2.15	.05
N	7.6	8.7	0.54	n.s.
S	2.4	1.9	2.64	.05
E	4.7	2.1	2.11	.05
A	1.30	1.09	2.11	.05

fitted to the normal curve (derived from data on the population on which the tests were standardized), they fall within normal limits.

DISCUSSION

During the 1920's and 1930's, psychiatric studies ascribed a causal role to the emotions in thyrotoxicosis. The person in whom thyrotoxicosis is likely to develop was characterized by Moschcowitz in 1930 (4): "In no instance were these patients normal . . . that which never disappears is the personality of the patient. . . . The patient with Graves' disease is never completely cured although he may be socially and economically restored."

Since 1940, a "classic" psychosomatic theory of the etiology of thyrotoxicosis has been evolved. As stated by Lidz (5, 6): "The patients have a single important interpersonal relationship and become ill at the time of its disruption or threat of disruption, an event which confronts them with their life-long fear of isolation from a protecting, mothering figure." They are thus left helpless, betrayed and enraged, becoming increasingly

TABLE 7. PERFORMANCE OF UNTREATED THYROTOXIC PATIENTS COMPARED WITH THAT OF PATIENTS EXHIBITING SCHIZOPHRENIC REACTIONS

Test	Patients with untreated thyrotoxicosis	Patients with schizophrenic reactions	*t*	*P*
C	15.5	23.5	2.42	.05
N	7.6	13.1	2.10	.05
S	2.4	2.5	0.32	n.s.
E	4.7	3.1	1.08	n.s.
A	1.30	1.47	1.29	n.s.

TABLE 8. PERFORMANCE IN UNTREATED THYROTOXIC PATIENTS COMPARED WITH THAT OF PATIENTS EXHIBITING STRUCTURAL CHANGES IN THE BRAIN

Test	Patients with untreated thyrotoxicosis	Patients with structural change in brain	*t*	*P*
C	15.5	17.7	0.57	n.s.
N	7.6	10.7	1.05	n.s.
S	2.4	2.7	0.92	n.s.
E	4.7	6.5	1.05	n.s.
A	1.30	1.26	0.38	n.s.

insecure, irritable and depressed. Hyperthyroidism may then occur. Recurrences depend on a similar psychologic setting.

Psychiatric probing of the personality of the thyrotoxic patient has been supported by results of psychologic tests oriented along psychoanalytical lines (7). These procedures were interpreted in the light of the psychodynamics implied by the patient's responses. Such tests, however, are not objective, are not free from error due to personal bias of the observer, and are not standardized (8).

There should be more emphasis on objective psychologic analysis of the emotional changes in thyrotoxicosis. With this in mind, we rejected the psychiatric interview and psychoanalytically oriented tests and chose objective techniques already proved by one of us (D.B.V.) to be of value in the differentiation of various emotional states. The tests to be used, we reasoned, should not be biased by the classic psychodynamic theory of thyrotoxicosis, however valid it might be, but should provide a different approach—one that could be quantitated and was as free as possible from intuitive interpretation. In this we have adopted the hypothetico-deduc-

TABLE 9. PERFORMANCE OF ADEQUATELY TREATED THYROTOXIC PATIENTS COMPARED WITH THAT OF CONTROLS

Test	Treated thyrotoxic patients	Normal controls	*t*	*P*
C	9.6	2.9	3.31	.01
N	6.0	3.8	1.37	n.s.
S	1.9	1.8	1.14	n.s.
E	1.8	1.1	0.82	n.s.
A	1.14	1.15	0.13	n.s.

TABLE 10. PERFORMANCE OF ADEQUATELY TREATED THYROTOXIC PATIENTS COMPARED WITH THAT OF PATIENTS EXHIBITING SOMATIZATION REACTIONS

Test	Patients with treated thyrotoxicosis	Patients with somatization reactions	*t*	*P*
C	9.6	16.9	2.65	.05
N	6.0	4.5	0.97	n.s.
S	1.9	2.0	0.63	n.s.
E	1.8	2.1	0.34	n.s.
A	1.14	1.06	0.78	n.s.

tive methods of Eysenck: "Objective measurement, appropriate techniques of statistical analysis and the realistic restriction of the effort of measurement to something less nebulous and less grandiose than the 'total personality'" (3). The 3 tests chosen for this pilot investigation are so simple that they may be given by a person with a minimum of training and interpreted without any knowledge of the patient by a psychologist, even in another city. Moreover, their use causes no harm as a result of interpretive probing by a psychiatrist.

SUMMARY

The role of the personality in thyrotoxicosis has been stressed, and classic psychodynamic studies have indicated that frustration of dependent needs is the predisposing factor in the onset of the disease. Our observations suggest that the role of the personality in thyrotoxicosis may have been overstressed. Patients with thyrotoxicosis were compared with normal subjects and other groups and the effect of treatment was observed. The tests we used differentiate between the factors of neuroticism and psy-

TABLE 11. PERFORMANCE OF ADEQUATELY TREATED THYROTOXIC PATIENTS COMPARED WITH THAT OF PATIENTS EXHIBITING OBSESSIVE COMPULSIVE REACTIONS

Test	Patients with treated thyrotoxicosis	Patients with obsessive compulsive reactions	*t*	*P*
C	9.6	22.1	5.11	.01
N	6.0	8.7	1.54	n.s.
S	1.9	1.9	0.34	n.s.
E	1.8	2.1	0.32	n.s.
A	1.14	1.09	0.61	n.s.

TABLE 12. PERFORMANCE OF ADEQUATELY TREATED THYROTOXIC PATIENTS COMPARED WITH THAT OF PATIENTS EXHIBITING SCHIZOPHRENIC REACTIONS

Test	Patients with treated thyrotoxicosis	Patients with schizophrenic reactions	*t*	*P*
C	9.6	23.5	5.15	.001
N	6.0	13.1	2.74	.05
S	1.9	2.5	2.54	.05
E	1.8	3.1	1.30	n.s.
A	1.14	1.47	2.74	.05

choticism; they also identify structural and/or physiologic changes in the nervous system associated with aging, disease or trauma. This approach, therefore, offers a decided advantage over the usual psychiatric interview and psychoanalytically oriented tests. We conclude that:

1. The presence and degree of thyrotoxicosis can be assessed by objective, psychologic techniques. There is a definite relationship between the clinical and psychologic manifestations of toxicity.
2. Using these techniques, the performance of untreated thyrotoxic patients is significantly different from that of normal controls, patients with somatization, and patients with obsessive-compulsive or schizophrenic reactions, but strongly similar to that of patients with organic structural changes in the brain.
3. There is no significant difference between the performances of adequately treated thyrotoxic patients, normal controls, and patients with somatization and obsessive-compulsive reactions.
4. Thyrotoxicosis is a metabolic disease manifested by impaired psychobiologic integration, involving particularly the autonomic nervous system. The adequately treated thyrotoxic patient closely resembles

TABLE 13. PERFORMANCE OF ADEQUATELY TREATED THYROTOXIC PATIENTS COMPARED WITH THAT OF PATIENTS EXHIBITING STRUCTURAL CHANGES IN THE BRAIN

Test	Patients with treated thyrotoxicosis	Patients with structural change in brain	*t*	*P*
C	9.6	17.6	2.44	.05
N	6.0	10.7	1.70	n.s.
S	1.9	2.7	2.70	.05
E	1.8	6.5	3.61	.01
A	1.14	1.26	1.31	n.s.

the normal person, who when under stress tends to internalize his conflicts and frustrations.

5. Thyrotoxicosis probably represents a toxic emotional phenomenon which overlies whatever happens to be the personality and experience pattern of the patient. Certain persons seem to have a predisposition to the disease. The emotional manifestations are correctable by adequate treatment of the somatic disorder, leaving the personality of the patient, as Moschcowitz has said, unchanged. In a susceptible patient, thyrotoxicosis may precipitate an inorganic, nontoxic psychosis which will not disappear along with improvement of the thyrotoxicosis.
6. Further studies are necessary in this area.

RECOMMENDATION

With this pilot study pointing a direction, it is possible to outline a more complete approach to the study of patients with thyrotoxicosis. The psychologic examination described here has the advantage of brevity and ease of administration and scoring. In no way is it a threat to the emotional adjustment of the thyrotoxic patient. However, it will be found lacking if one wishes to investigate autonomic imbalance per se, or to test psychoanalytic hypotheses. It would be worth while to study the complaints of the thyrotoxic patients in relation to conditioning experiences, to utilize objective measures of autonomic function as described by Wenger (9), and to include tests which assess the changes in psychobiologic integration associated with aging, disease or trauma (2).

REFERENCES

1. Ham, G. C.; Alexander, F., and Carmichael, H. T.: A psychosomatic theory of thyrotoxicosis, *Psychosom. Med.* **13**: 18–35 (Jan.-Feb.) 1951.
2. Vinson, D. B.: Psychobiological decline, *Internat. Rec. Med.* **165**: 268–272 (May) 1952.
3. Eysenck, H. J.: The Dynamics of Anxiety and Hysteria. London, Routledge and Kegan Paul, 1957.
4. Moschcowitz, E.: The nature of Graves' disease, *Arch. Int. Med.* **46**: 610–629 (Oct.) 1930.
5. Litz, R.: Emotional factors; emotions and mentation, *in* The Thyroid, ed. by S. C. Werner. New York, Hoeber-Harper, 1955, pp. 448–453 and 573–575.
6. Lidz, T.: Emotional factors in the etiology of hyperthyroidism, *Psychosom. Med.* **11**: 2–8 (Jan.-Feb.) 1949.
7. Bartels, E. C.: Heredity, *in* The Thyroid, ed. by S. C. Werner. New York, Hoeber-Harper, 1955, pp. 440 ff.
8. Garner, A. M.: Abnormalities of behavior, *Ann. Rev. Psychol.* **9**: 391–418, 1958.
9. Wenger, M. A.: Preliminary study of the significance of measures of autonomic balance, *Psychosom. Med.* **9**: 301–309 (Sept.) 1947.

MULTICENTRICITY OF PAPILLARY ADENOCARCINOMA OF THE THYROID: INFLUENCE ON TREATMENT*

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ABSTRACT

In a study of 328 cases of papillary adenocarcinoma of the thyroid treated surgically in the period 1940–1954, it was found that these adenocarcinomas are multicentric in at least 20 per cent of cases. The multicentricity usually involves both lobes. Given a papillary lesion in one lobe, one or more additional deposits occur in the same lobe in more than 10 per cent of cases, and in the opposite lobe in a like proportion of cases. Further resection of the thyroid is advisable in cases in which partial lobectomy or partial thyroidectomy has been carried out previously because of a nodule that subsequently proves to be a papillary lesion. Preservation of a remnant on the apparently uninvolved side together with the nodes in the tracheo-esophageal groove on that side is justified in order to avoid total parathyroidectomy.

IN VIEW of its obvious importance, particularly from the standpoint of treatment, multicentricity of papillary lesions of the thyroid has received surprisingly little special attention. Sloan (1) found 30 cases of multicentricity in a series of 282 papillary, follicular, mixed papillary and follicular and Hürthle-cell tumors (his group I tumors). In patients from 4 to 25 years of age, 12 of 39 tumors were multiple, whereas only 18 of 243 lesions in patients older than 25 were multicentric. A similar incidence was reported by Horn and Dull (2) who found 15 instances in a series of 115 cases. The multiple foci were evident grossly in 9 cases but in 6 they were found only on meticulous microscopic examination.

A materially higher incidence was reported by Underwood, Ackerman and Eckert (3). Some part of both lobes of the thyroid was available for pathologic examination in 25 of 62 cases of papillary adenocarcinoma. At

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least two foci of cancer were found in 8 of the 25. The authors speculated that the incidence would have been higher if serial sections had been employed and if the entire thyroid could have been examined. Multicentricity has been mentioned, and an occasional case cited, by others, but no other studies of the incidence of the condition have appeared in the recent literature (4–6).

PRESENT STUDY

In an effort to arrive at another estimate of the frequency of multicentricity, all cases of papillary adenocarcinoma of the thyroid treated at the Mayo Clinic from 1940 through 1954 were reviewed. Cases in which a biopsy specimen had been removed from the thyroid, or in which a part of the thyroid had been excised previously, were excluded from the study. Predominantly papillary ("pure papillary lesions"), mixed papillary and follicular, and predominantly follicular lesions were included. Thyroidectomy had been advised in the great majority of cases because of one or more nodules within the thyroid. The diagnosis had been established previously in 45 cases by biopsy of a cervical lymph node.

Serial sections were not employed. The surgical specimens were examined, however, by pathologists quite cognizant of the possibility of multicentricity. The foci of papillary adenocarcinoma varied greatly in both size and number from case to case. Occasionally the lesions were small and unexpected clinically, whereas in other cases two or more sizable foci were found. Far more commonly, one lesion was large enough to be evident on clinical examination while the others were substantially smaller, often measuring not more than a few millimeters in diameter.

During the interval represented by the study, the extent of resection of the thyroid customarily carried out in the treatment of papillary lesions increased considerably. Unilateral subtotal lobectomy was often believed adequate during the earlier years, provided the surgeon, knowing the diagnosis, thought that the lesion had been removed completely. The opposite lobe was not disturbed if it appeared normal grossly. After 1945, the involved lobe was removed totally, as a rule, and since about 1950 the opposite lobe was resected subtotally as well. In operable cases, total thyroidectomy was carried out only in instances of marked multicentricity or when the remnant on the opposite side appeared grossly to be involved.

The multicentricity found in the series of 328 cases of papillary adenocarcinoma treated during the 15-year period is shown in Table 1. The findings obviously should be regarded as representing a minimal proved incidence rather than the true incidence of multicentricity. The over-all incidence of 12 per cent is clearly too low, since the second lobe was not available for pathologic examination in 186 cases and since the entire

thyroid was examined in only 10 cases. The incidence of 50 per cent in the 10 cases in which total thyroidectomy was carried out is probably far too high in that the indication for the procedure was often known multicentricity. In the 142 cases in which both lobes were removed totally or partially, multicentricity was found in 26 or in 18 per cent. This is probably the best estimate of multicentricity provided by the pathologic findings alone.

At least one additional focus was present in the same lobe as the clinically evident primary in 39 of the 328 cases (12 per cent). Somewhat unexpectedly, the same incidence (26 of 209 cases, or 12 per cent) was found in

TABLE 1. MULTICENTRICITY OF PAPILLARY ADENOCARCINOMA OF THE THYROID: OPERABLE, PREVIOUSLY UNTREATED PATIENTS (1940–1954)

Surgical procedure	Total cases	Cases of multicentricity				
		Total		Same lobe	Opposite lobe	Lobe not known
		Number	Per cent			
Unilateral subtotal lobectomy	69	4	6	4	—	—
Bilateral subtotal lobectomy	50	8	16	6*	5*	2
Total lobectomy	117	9	8	9	—	—
Total lobectomy and contralateral subtotal lobectomy	82	13	16	12†	9†	1
Total thyroidectomy	10	5	50	5†	3‡	—
Total	328	39	12	36	17	3

* Both lobes involved, 5 cases.
† Both lobes involved, 9 cases.
‡ Both lobes involved, 3 cases.

cases in which the entire lobe was available for study. In the 142 cases in which all or part of the opposite lobe was available for pathologic study, at least one deposit was found in 17, or again in 12 per cent of cases. In every case in which there were deposits in the opposite lobe, there was also multicentricity in the lobe that harbored the clinically evident primary.

The known recurrences in the preserved remnants, or what Rundle and Basser (6) have called "stump recurrences," are shown in Table 2. Although 184 patients had been treated at least five years before the date of inquiry, 22 had been lost to follow-up or had died within five years, leaving 162 patients traced for five to fifteen years. Recurrence in unresected thyroid tissue in 10 per cent of cases demonstrates clearly the inadequacy of the earlier routine resections of the gland. With respect to the incidence

of multicentricity, recurrence in the lobe that harbored the clinically evident primary tumor has little meaning, since in many cases of recurrence the lesion was known to have been multicentric. The recurrences could have resulted also from failure to remove the known lesion completely. In the 123 cases in which the opposite lobe was not resected, the 10 recurrences in that lobe (8 per cent) must have developed from multicentric foci. Although all were not additional cases of multicentricity, it is of interest that the 8 per cent rate added to the 12 per cent rate found on pathologic examination for the group as a whole yields an incidence wholly in keeping with the 18 per cent rate mentioned previously.

TABLE 2. RECURRENCE OF PAPILLARY ADENOCARCINOMA IN THYROID REMNANT (5 TO 15-YEAR FOLLOW-UP)

Operative procedure	Total cases	Cases of recurrence				
		Total		Same lobe	Opposite lobe	Lobe not known
		Number	Per cent			
Unilateral subtotal lobectomy	60	8*	13	4	5	—
Bilateral subtotal lobectomy	26	4*	15	2	2	1
Total lobectomy	63	5	8	—	5	—
Total lobectomy and contralateral subtotal lobectomy	12	—	—	—	—	—
Total thyroidectomy	1	—	—	—	—	—
Total	162	17	10	6	12	1

* Bilateral recurrence in 1 case.

The findings may be summarized briefly as follows: Papillary lesions are multicentric in at least 20 per cent of cases. The multicentricity usually involves both lobes of the thyroid. Additional deposits of cancer may be expected in the same lobe as the clinically evident primary lesion in more than 10 per cent of cases, and in the opposite lobe in a similar proportion of cases.

Although we have no particular opinion as to whether the multiple foci represent true multicentricity or intrathyroidal metastatic growths, the findings favor the former view. If they were metastatic lesions, more deposits would be expected in the lobe that harbors the larger lesion than in the opposite lobe, and there should be some pattern of spread discernible within the thyroid. As noted previously, multicentricity involved both lobes equally, and the multiple lesions seemed to have no particular distribution within the thyroid.

COMMENT

Our present interest in multicentricity was actually stimulated by an increasingly large number of patients referred for consideration of further treatment after removal of an unsuspected papillary lesion elsewhere. In the usual case the lesion had been removed locally or, at most, a subtotal thyroidectomy had been performed. Formerly, providing the surgeon thought that the lesion had been removed completely, continued observation alone was advised. As our own practice changed toward more radical resections, it seemed logical to propose that further resection should be advised in such cases.

On this basis a more radical resection of the thyroid was carried out at the clinic in 17 consecutive cases in which subtotal lobectomy or subtotal thyroidectomy had been performed elsewhere within six months. Residual or multicentric carcinoma was found in the preserved part of the thyroid in 11 cases. In view of this and of more recent experience, and in keeping with the practice of some others (7), we now believe that the thyroid should be re-explored in all such cases in which less than a bilateral resection with total lobectomy on the side of the clinically evident nodule was carried out. In actual practice re-exploration is delayed for about three months to permit the inflammatory reaction resulting from the original procedure to subside.

For some ten years, routinely, the obviously involved lobe has been removed totally and the opposite lobe resected subtotally in the treatment of papillary lesions. More accurately, the usual bilateral subtotal thyroidectomy is carried out, due care being exercised not to cut into any suspicious nodule. The incision is not closed until the report of the pathologist has been received. If a papillary lesion is found, the remnant on that side is removed, the nerve being preserved unless it is apparently involved. The remnant on the opposite side is preserved as a rule. Total thyroidectomy is carried out only in cases of marked multicentricity and in those with large lesions in both lobes. Needless to add, total thyroidectomy may be indicated in anticipation of treatment of inoperable lesions with radioiodine.

Discussion of the treatment of cervical lymph nodes is beyond the scope of the present paper. The treatment of nodes in the tracheo-esophageal groove, however, can well be considered in conjunction with resection of the thyroid. The juxtathyroidal nodes are unquestionably involved more frequently than the deep jugular nodes, and they are not accessible to clinical examination as are the lateral cervical nodes. Consequently, we believe that the nodes and node-bearing fascia should be removed cleanly from the tracheo-esophageal groove and from the region of the recurrent

laryngeal nerve on the side of the obviously involved lobe in all cases. The nodes in the opposite tracheo-esophageal groove should be inspected most carefully. We do not believe that they should be removed unless they are obviously involved, or unless total thyroidectomy is thought necessary.

Our conservatism regarding the second remnant and the nodes in the opposite tracheo-esophageal groove stems wholly from the desire to avoid permanent tetany. Of the 10 cases in the present series in which total thyroidectomy and bilateral resection of paratracheal nodes were carried out, permanent tetany ensued in 5. In a different series of total thyroidectomies reported by Beahrs and his colleagues (8) from the clinic, tetany developed in 13 of 27 cases. Thus, in our experience, tetany develops in about half of all cases in which total thyroidectomy with removal of nodes from both tracheo-esophageal grooves is carried out. Tetany ensues in spite of efforts to preserve at least one parathyroid with an intact vascular supply, and regardless of whether parathyroids are implanted into regional muscles. We have no evidence that devascularized parathyroids, whether left in situ or implanted in muscles, ever survive and function. Conversely, saving devascularized parathyroids has failed to prevent tetany repeatedly.

REFERENCES

1. Sloan, L. W.: Of the origin, characteristics and behavior of thyroid cancer, *J. Clin. Endocrinol. & Metab.* **14:** 1309 (Nov.) 1954.
2. Horn, R. C., Jr., and Dull, J. A.: Carcinoma of the thyroid: a re-evaluation, *Ann. Surg.* **139:** 35 (Jan.) 1954.
3. Underwood, C. R.; Ackerman, L. V., and Eckert, C.: Papillary carcinoma of the thyroid: an evaluation of surgical therapy, *Surgery* **43:** 610 (Apr.) 1958.
4. Klinck, C. H., and Winship, T.: Occult sclerosing carcinoma of the thyroid, *Cancer* **8:** 701 (July–Aug.) 1955.
5. MacDonald, I., and Kotin, P.: Surgical management of papillary carcinoma of the thyroid gland: the case for total thyroidectomy, *Ann. Surg.* **137:** 156 (Feb.) 1953.
6. Rundle F. F., and Basser, A. G.: Stump recurrence and total thyroidectomy in papillary thyroid cancer, *Cancer* **9:** 692 (July–Aug.) 1956.
7. Frazell, E. L., and Foote, F. W., Jr.: Papillary cancer of the thyroid: a review of 25 years of experience, *Cancer* **11:** 895 (Sept.–Oct.) 1958.
8. Beahrs, O. H.; Ryan, R. F., and White, R. A.: Complications of thyroid surgery, *J. Clin. Endocrinol. & Metab.* **16:** 1456 (Nov.) 1956.

The Endocrine Society

The 1960 Annual Meeting

The Forty-Second Annual Meeting of The Endocrine Society will be held in the Eden Roc Hotel, Miami Beach, Florida, Thursday, Friday and Saturday, *June 9, 10 and 11, 1960.*

The Chairman of Local Arrangements is Dr. Kenneth Savard.

Scientific Sessions will be held from 9:00 A.M. to 5:00 P.M. daily and in addition there will be simultaneous afternoon sessions. The annual dinner is scheduled for Friday, June 10, at 7:30 P.M. preceded by cocktails at 6:30 P.M.

All members are urged to make their hotel reservations immediately. The Eden Roc will hold 250 bedrooms and the adjoining hotels, the Montmartre Hotel and Lucerne Hotel, will hold 75 rooms each for members until May 1, 1960, after which time the hotel will not guarantee further reservations. Therefore it is imperative that you make your reservations early, directly with the hotel, advising them of time and date of arrival and departure. If you plan on remaining through June 12 for the American Medical Association and other meetings, it is imperative that your reservation be made from June 12 on, directly through the A.M.A. Housing Bureau, c/o Miami Beach Convention Bureau, 1700 Washington Avenue, Miami Beach, Florida, since no reservation beyond June 12 can be accepted directly by the hotels. Make your reservations early and avoid disappointment. Hotel reservation card will be mailed to members shortly after January 1, 1960.

Final program, membership card and advance registration forms will be sent on May 1, 1960 to members whose current dues have been paid.

Those wishing to present papers, which will be strictly limited to ten minutes, should send an original and 4 copies of the title and abstract to the Vice-President, Dr. Robert H. Williams, University of Washington School of Medicine, Seattle 5, Washington, *not later than February 15, 1960.* It is imperative that the abstracts be informative and complete with results and conclusions—not a statement that these will be presented at the meeting—in order that they may be of reference value and suitable for printing in the program. The reading and processing of approximately 200 abstracts submitted each year and compiling of the program from these

involves a tremendous amount of time and effort on the part of the Program Committee. THE COUNCIL REQUESTS THAT AUTHORS ADHERE STRICTLY TO THE FOLLOWING RULES WHEN SUBMITTING ABSTRACTS; OTHERWISE THEY CANNOT BE CONSIDERED:

1. IT IS ASSUMED THAT ABSTRACTS SUBMITTED FOR THIS PROGRAM HAVE NOT AND WILL NOT BE SUBMITTED ELSEWHERE.
2. Abstracts may not exceed 200 words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formula cannot be used.
3. The title heading must be arranged as follows:
   Line 1. Title, not to exceed 15 words;
   Line 2. Author/s. The name of each nonmember-author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of nonmembers who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be written after his name.
   Line 3. Institution of origin and city in which institution is located.
4. Authors who wish receipt of their abstracts acknowledged should enclose self-addressed post card with the title of the abstract noted thereon.
5. Authors are also to enclose 3×5 inch filing cards, one for each author, giving his name and initials in the upper right-hand corner, and the complete title of the abstract with all the authors listed in the center of the card. There should be a second card enclosed listing the title, all authors, and the mailing address of the senior author. This is necessary for indexing. Senior authors will be notified of the acceptance of their papers for the final program.
6. The body of the abstract, typed double space, should follow the heading. The original copy should be on bond paper. The original and 4 copies should be forwarded to Dr. Williams.
7. Abstracts should be letter perfect, since there will be no opportunity for proof reading by the authors.

The 1960 Awards

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

Fred Conrad Koch Award

In 1957 a substantial legacy was bequeathed to the Society by the late Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service Professor of Physiological Chemistry at the University of Chicago and pioneer in the isolation of the androgens. This is the highest honor of The Endocrine Society and is represented by a medal known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award was first presented in 1959, to Dr. W. W. Swingle. The Koch Medal has replaced the Medal of The Endocrine Society which was established in 1954 and presented to Dr. Carl R. Moore in 1955, Dr. Frederick L. Hisaw in 1956, Dr. Joseph C. Aub in 1957 and Dr. I. L. Chaikoff in 1958. The Medal of the Endocrine Society replaced the E. R. Squibb Award which was formerly the highest honor bestowed by the Society. Past recipients of the Squibb Award were Dr. George W. Corner in 1940, Dr. Philip E. Smith in 1941, Dr. Fred C. Koch in 1942, (no award in 1943), Dr. E. A. Doisy in 1944, Dr. E. C. Kendall in 1945, Dr. Carl G. Hartman in 1946, Drs. Carl F. and Gerty T. Cori in 1947, Dr. Fuller Albright in 1948, Dr. Herbert M. Evans in 1949, Dr. C. N. H. Long in 1950, Dr. J. B. Collip in 1951, Dr. James H. Means in 1952 and Dr. David Marine in 1953.

The Ciba Award

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—

Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi; 1958—Dr. Monte A. Greer; 1959—Dr. Gordon L. Farrell. Prior to 1952 the Award was $1,200. It has now been increased to $2,500.

The Ayerst and the Squibb Fellowships

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

The Upjohn Scholar of the Endocrine Society

The Council of The Endocrine Society has established a category of Scholars. A grant has been made available through the generosity of the Upjohn Company, and will be awarded to an established investigator or teacher in the field of endocrinology who wishes to extend the opportunities for work either in this country or abroad.

The award will not exceed $2,500 annually and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be

obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15* each year.

Proposals for appointments as a Scholar of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15* each year.

First International Congress of Endocrinology

The First International Congress of Endocrinology will be held in *Copenhagen, Denmark, July 18–23, 1960.*

United States members of the International Executive Committee are Dr. Warren O. Nelson and Dr. Leo T. Samuels, Dr. Gregory Pincus is Chairman of the Program Committee. The tentative program consists of ten symposia covering the general field of endocrinology plus round-table discussions and a number of submitted papers. Those desiring to submit papers should immediately contact Dr. Gregory Pincus, Worcester Foundation, Shrewsbury, Massachusetts. There will be a number of interesting scientific and technical exhibits.

In order to facilitate travel arrangements to the Copenhagen Convention, the Council of The Endocrine Society at its San Francisco meeting designated THOMAS COOK & SON as our authorized travel agents. They will in turn offer especially designed All Expense Paid Tours to Europe, including hotel reservations for the convention, as well as offering independent itineraries for any of our members who desire to travel separately by air or by steamship.

Registration is open to anyone interested in endocrinology at an approximate fee of $28 U.S. Members of the families of those attending may register as associates at an approximate fee of $14 U.S., fee to be paid at the time of registration or in advance to the American Express Company, which has been designated by the Executive Committee of the International Congress as official financial and travel agent. There will be a number of social functions and other entertainment provided for all registrants.

As you all know, European travel is unusually heavy during July and it is suggested that those contemplating attending the Congress should start making tentative reservations at the earliest possible moment by filling in and returning the card recently mailed to members, to THOMAS COOK & SON's special representative—Harry E. Kornbaum, Transportation Chairman, Rainbow Travel Service, 2817 Classen Boulevard, Oklahoma City 6, Oklahoma, who will correspond directly with each member about his individual travel arrangements to and from the Convention, at the same time keeping our National Officers fully informed of all travel arrangements

of its members. Completion of the card CARRIES NO OBLIGATION OR COMMITMENT ON YOUR PART. Send it in today for the complete travel arrangements for our Society.

The general expenses of a meeting of this magnitude are quite large and The Endocrine Society has made a substantial contribution from its treasury for organizational purposes. U.S. pharmaceutical firms have also contributed very generously. However, sufficient funds are not as yet available to assure the success of this meeting; therefore, *the committee suggests that each of our members make voluntary contributions to this fund.* Please make your checks payable to: The Endocrine Society—First International Congress of Endocrinology. Mark it *Contribution* and mail it to the office of the Secretary-Treasurer, 1200 North Walker Street, Oklahoma City 3, Oklahoma.

Program

The program of the First International Congress of Endocrinology which will be held in Copenhagen, Denmark, July 18–23, 1960, will consist of ten symposia and a round table discussion, along with sessions of communicated papers. The speakers at the symposia and the round table discussion have been invited by the Subcommittee on Program of the Congress. In Symposia #1–8 and in #10, the Subcommittee has also invited a discusser for each principal paper. Symposium #9 will consist of six principal papers and no discussers. The round table discussion will consist of eight sections, each with a principal speaker and three secondary speakers.

(For program see the December 1959 issue of the Journal.)

The American Goiter Association

1960 Annual Meeting and the 4th International Goiter Conference

The next Annual Meeting of the American Goiter Association will be held in *London, England, July 5–9, 1960,* in the Great Hall of the Royal College of Surgeons. This meeting, co-sponsored with the London Thyroid Club, will be the Fourth International Goiter Conference. For further information write Dr. Selwyn Taylor, 3 Roedean Crescent, Roehampton, London, S.W.15, England.

John C. McClintock, *Secretary,*
149½ Washington Avenue,
Albany 10, N.Y.

Hormones Available—National Institutes of Health

Group A. The following highly purified pituitary hormones are available for distribution free to qualified investigators:

Growth hormone, bovine, non-sterile for animal experiments only. Dispensed as requested.

Follicle-stimulating hormone, ovine, sterile preparation: 25-mg. vials for experiment; 5-mg. vials for assay standard.

Luteinizing hormone, ovine, sterile preparation: 10-mg. vials.

Prolactin, ovine, sterile preparation: 25-mg. vials.

Workers may obtain supplies of these hormones by writing to: Dr. R. T. Hill, Executive Secretary, Endocrinology Study Section, Division of Research Grants, National Institutes of Health, Bethesda 14, Maryland, describing briefly the proposed work and giving an estimate of the amount of hormone required. Requests are filled as promptly as possible after review by a subcommittee. These hormones are prepared and distributed under a program generously supported by the National Institutes of Health.

Group B. The following radioactive steroids are available without charge for materials, upon receipt of an adequate résumé of proposed experiments:

	Material	Batch	Specific activity
1.	Hydrocortisone 4-C^{14}	ES-F-10-1	8.7 μc./mg.
	Hydrocortisone 4-C^{14}	ES-F-25-1	21.7 μc./mg.
	Hydrocortisone 4-C^{14}	ES-F-50-1	42.4 μc./mg.
2.	Corticosterone 4-C^{14}	ES-B-10-1	9.1 μc./mg.
	Corticosterone 4-C^{14}	ES-B-50-1	44.4 μc./mg.

These materials are stored at Tracerlab, Inc., Waltham, Massachusetts. They are dispensed in sealed glass ampules containing 5 μc., 10 μc. or 25 μc. each, and in a solution of 95% benzene and 5% methanol. Shipments will be made from Tracerlab, Inc., and the recipient will be billed by them, at the rate of $5.00 per ampule for handling and mailing. Requests for these compounds should be submitted to: Dr. R. T. Hill, Executive Secretary, Endocrinology Study Section, Division of Research Grants, National Institutes of Health, Bethesda 14, Maryland. The materials are approximately 96% pure, and each investigator is cautioned to calibrate each batch of material used.

New York University Post-Graduate Medical School

announces the following post-graduate course 563R in

GYNECOLOGICAL ENDOCRINOLOGY

A practical, didactic and clinical presentation with emphasis on the therapeutic management of endocrine disorders in the female, including a discussion of the diagnosis and management of intersex will be offered. Endocrine therapy for menstrual abnormalities and ovulatory defects will be presented. Adrenocortical, thyroid, ovarian and pituitary abnormalities will be emphasized. Practical diagnostic tests will be discussed and demonstrated, as well as the therapeutic use of the newer progestational, gonadal and corticoid steroids. Five-day, full-time course, *April 18 through April 22, 1960.* Tuition: $140.00. Given under the direction of Dr. Herbert S. Kupperman. For further information: Office of the Associate Dean, New York University Post-Graduate Medical School, 550 First Avenue, New York 16, N. Y.

University of Oklahoma Colloquy on Advances in Internal Medicine

The Third Annual Oklahoma Colloquy on Advances in Medicine will be held *March 24–26, 1960,* at the University of Oklahoma Medical Center, Oklahoma City, with a program devoted to the *adrenal steroids.*

Fifteen guest lecturers and 13 members of the Medical Center faculty will present papers dealing with the basic physiological and biochemical aspects of adrenal steroids. The pharmacology and side-effects will be discussed.

Scientific reports also will cover clinical application of the steroids in the following areas: endocrinology, infectious diseases, gastroenterology, rheumatic disease, hematological diseases (including the leukemias and immuno-hematological disorders), neoplastic, collagen, renal and allergic diseases.

In addition to general sessions, special interest meetings are planned in the fields of medicine, pediatrics, surgery and basic science.

The Colloquy was developed by the Oklahoma Medical Center Department of Medicine and Office of Postgraduate Education in collaboration with the Oklahoma City Internists Association, Oklahoma City Surgical Society and the Oklahoma City Pediatric Society. Merck Sharp & Dohme is a sponsor.

Additional information may be obtained by writing the Office of Post-

graduate Education, University of Oklahoma Medical Center, 801 NE 13, Oklahoma City, Oklahoma.

Symposium on Uterine Implantation of Ova

A Symposium devoted entirely to problems relative to the uterine implantation of ova, in animals and in the human being, will be held in Brussels, Belgium, *June 24–26, 1960*, just before the First International Congress of Endocrinology in Copenhagen. It is being organized jointly by the Société Nationale Française pour l'Etude de la Stérilité et de la Fécondité (Président: Professeur P. Funck-Brentano) and the Belgian Section of the International Fertility Association. All interested persons are invited to attend. The working languages will be English and French, with simultaneous translation.

Registration: Dr. P. Wilkin, 75, rue Général Lotz, Bruxelles, Belgium. Registration fee (volumes of the Proceedings included) $20.00, Scientific information: Dr. J. Ferin, 42, Beukenlaan, Heverlee-Louvain, Belgium.

BOOK REGISTER

(*The*) *Diabetic's Handbook*. Second edition. By ANTHONY M. SINDONI, JR., M.D., Chief of the Department of Metabolism, St. Joseph and Philadelphia General Hospitals, Philadelphia, Pennsylvania, with 18 collaborators. In simple language, this handbook outlines the knowledge necessary to help the diabetic and his family live with his condition. Seven chapter headings are: Questions the Diabetic Asks, and the Physician's Answers; Causes, Symptoms and Diagnosis of Diabetes; Complications of Diabetes; Insulins and Oral Medication; Foods; Laboratory Tests and General Hygiene; and The Present and Future for the Diabetic; 286 pages; 1959. The Ronald Press Company, New York, N.Y. Price $4.50.

Diagnosis and Treatment of Menstrual Disorders and Sterility. Fourth edition of Mazer & Israel's book by S. LEON ISRAEL, M.D., Professor of Gynecology and Obstetrics, Graduate School of Medicine, University of Pennsylvania; Chief Gynecologist, Graduate Hospital; Gynecologist and Obstetrician, Pennsylvania Hospital, Philadelphia, Pennsylvania. This well known textbook has been almost completely rewritten in order to reflect the progress of recent years, and is intended as a guide for the family physician in treating the numerous cases of menstrual disorders and sterility that he will inevitably encounter in a busy practice. There are three new chapters on the role of the thyroid, the adrenal cortex, and androgens respectively. New procedures and treatment are discussed. The chapter on the male factor in sterility has been rewritten. The more pertinent findings of fundamental scientists have been interpreted in the light of their useful applications. Many bibliographic references; 666 pages; 147 illustrations; 1959. Paul B. Hoeber, Inc., New York, N.Y. Price $15.00.

Endocrinología. Edition 5. By DR. RODOLFO Q. PASQUALINI, Jefe del Servicio de Endocrinología del Hospital Militar Central; Ex Profesor Titular de Clinica Médica y del Curso de Endocrinología para Graduados de la Facultad de Medicina de

Buenos Aires; Ex Director del Instituto Nacional de Endocrinología, Buenos Aires, Argentina. After opening chapters on the structure of the endocrine glands and on the individual hormones, the book covers the subject of clinical endocrinology in detail, including diagnosis and treatment. The author has attempted to strike a balance between the physiological and the clinical, the classical and the modern, the usual and the exceptional, and between theory and practice. Argentinian contributions to the fundamental and clinical aspects of endocrinology are included. There are over 400 illustrations; 866 pages (in Spanish); 1959. El Ateneo, Buenos Aires, Argentina.

Fermente, Hormone, Vitamine und die Beziehungen dieser Wirkstoffe zueinander. Edition 3. By PROF. DR. ROBERT AMMON, Physiologisch-Chemisches Institut der Universität des Saarlandes, Homburg/Saar and PROF. DR. WILHELM DIRSCHERL, Physiolgisch-Chemisches Institut der Universität, Bonn/Rhein. *Band I. Fermente.* Ed. by R. AMMON, Homburg/Saar and K. MYRBÄCK, Institutet för organisk kemi och biokemi, Stockholm Va. The volume has been brought up to date and contains chapters on the general and special aspects of ferments (enzymes) that will be of particular interest to biochemists. The chapters cover the hydrolases, carbohydrases, C-N-hydrolases (amidases and proteases), transferases, isomerases, lyases (aldolase, etc.), enzymatic decarboxylation, biologic oxidation, anaerobic carbohydrate reduction, the citric acid cycle, oxidative phosphorylation, the fatty acid cycle and photosynthesis; 564 pages; 34 illustrations; 53 tables; 1959. Georg Thieme Verlag, Stuttgart, Germany. Subscription price, DM 81.60 ($19.45). In U.S.A. and Canada, Intercontinental Medical Book Corporation, New York, N.Y.

Medical Management of the Menopause. A Modern Medical Monograph. By MINNIE B. GOLDBERG, M.D., Associate Clinical Professor of Medicine, University of California School of Medicine, San Francisco, California. The author's clinical experience covers both the prehormonal and hormonal eras and has convinced her of the value of hormonal therapy. The menopause is discussed in chapters covering the physiology of menstruation and the etiology of the menopausal syndrome, the physiologic, metabolic and pharmacologic aspects of the gonadal steroids, symptomatology of the menopause, diagnosis and diagnostic procedures, therapeutic management, hormonal and hormone-like agents for treatment, and problems in therapy; 98 pages; 10 figures; 5 tables; 1959. Grune & Stratton, New York, N.Y. Price $4.50.

Metabolic Homeostasis. A Syllabus for Those Concerned with the Care of Patients. By NATHAN B. TALBOT, M.D., Assoc. Professor of Pediatrics, Harvard Medical School; Physician, Children's Medical Service, Massachusetts General Hospital, Boston, Mass.; ROBERT H. RICHIE, M.D., Instructor in Pediatrics, Harvard Medical School; Assistant Physician, C.M.S., Massachusetts General Hospital; JOHN D. CRAWFORD, M.D., Assistant Professor of Pediatrics, Harvard Medical School; Physician, C.M.S., Massachusetts General Hospital; and EDITH S. TAGRIN, Medical Artist, Massachusetts General Hospital. Information regarding the manner in which the body maintains a steady state through suitable adjustments is applied to the diagnostic and therapeutic problems that arise in the care of patients, The clinically useful form in which this information is presented enables the physician to become familiar with the limits of capacity of the body's homeostatic systems. Section A (Basic Considerations) deals with the water, the sodium and the hydrogen ion homeostatic

systems, and chloride and phosphorus homeostasis. Section B (Clinical Applications) deals with infant feeding, parenteral fluid therapy, and renal disabilities. An important book concerned with fundamental concepts. There are several convenient charts for converting various terms of measurement; 134 pages; 47 figures; 4 graphs; 1959. Cambridge, Mass., Harvard University Press.

(*Die*) *Sterilität der Frau. Ein Leitfaden der Diagnostik und Therapie für die Praxis.* By PROF. DR. WERNER BICKENBACH, Direktor, I. Universitsäts-Frauenklinik München, and DR. GERD K. DÖRING, Privatdozent für Frauenheilkunde an der Universität, München, Germany. Recent advances in the diagnosis and management of sterility are discussed from the viewpoints of constitution, genital organs, control of ovarian function, spermatic function in the male, tubal patency, pituitary, ovarian, tubal, uterine, cervical, vaginal and various extragenital causes of sterility, and artificial insemination; 71 pages; 1959. Georg Thieme Verlag, Stuttgart, Germany. Price DM 6.80.

Tumors of the Pancreas. Section VII—Fascicles 27 and 28, Atlas of Tumor Pathology. By VIRGINIA KNEELAND FRANTZ, M.D., Professor of Surgery, College of Physicians & Surgeons, Columbia University; Attending Surgical Pathologist, Presbyterian Hospital, New York, N.Y. Published by the Armed Forces Institute of Pathology under the auspices of the Subcommittee on Oncology of the Committee on Pathology of the Division of Medical Sciences of the National Academy of Sciences—National Research Council. In this well documented and illustrated volume, tumors of the pancreas are dealt with under the following headings: Anatomy, heterotopia, tumors of pancreatic tissue, exclusive of islet-cell neoplasms (cysts, benign neoplasms of exocrine glands, carcinoma of exocrine glands, and rare malignant tumors other than primary carcinoma), islet-cell tumors (benign, malignant), lesions associated with islet-cell tumors (including tumors of multiple endocrine organs), and extrapancreatic tumors associated with hypoglycemia; 149 pages; 94 figures; 1 color plate; 3 tables; 1959. American Registry of Pathology, Armed Forces Institute of Pathology, Washington 25, D. C. Price $1.50.